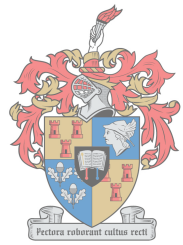
Fabrication and characterisation of poly (ethylene-co-vinyl alcohol) nanofibers with biocidal additive for water filtration

By

Margaret Motsi



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Declaration

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Margaret Motsi March 2018

Abstract

Water is an undeniable right for all humans and their wellbeing is dependent upon equitable access to a safe, adequate and affordable water supply. The world is facing formidable challenges in meeting rising demands of clean water as the available supplies of fresh- water are decreasing due to (i) extended droughts, (ii) population growth, (iii) more stringent health-based regulations, and (iv) competing demands from a variety of users.

The aim of this study is to prepare and compare three types of poly (ethylene-co-vinyl alcohol) (EVOH) nanofibers and determine which type of fibre will be most suitable for the application in water sanitation. Testing of antimicrobial activity of the AquaQure containing EVOH nanofibers, as well as leaching out of metal ions which could imply toxicity and reusability of the EVOH nanofibers modified with the AquaQure biocide will be investigated.

As an effort to do away with the conventional methods of water purification that involve the use of chemicals which introduce harmful substances to the environment, fabrication and characterization of anti-microbial polymer nanofibers with a nanobiocide will be done in this study. Secondly, the problem of biofouling on filtration membranes will be addressed by fabricating and testing polymer nanofibers with different degrees of hydrolysis.

To address water sanitation, three types of EVOH nanofibers, (i) 27 mol. % (ii) 32 mol. % and (iii) 44 mol. % ethylene content are to be prepared using single needle electrospinning. AquaQure an aqueous antimicrobial agent containing mainly Cu^{2+} and Zn^{2+} ions will be used as an additive to the polymer solutions.

The nanofibrous mats, neat EVOH and modified EVOH/AquaQure were successfully fabricated using the conventional single needle electrospinning. The incorporation of the biocide was confirmed by EDX, ATR-FTIR, TGA, DSC and SEM techniques were used to do chemical and thermal analysis of the nanofibers in comparison with neat EVOH nanofibers. The hydrophobicity of nanofibrous mats was measured using static contact angle measurements.

The testing of antimicrobial activity of the AquaQure containing EVOH nanofibers against the gram positive and gram negative bioluminescent strains of *Staphylococcus aureus* Xen-36 and *Escherichia coli* Xen-14 respectively. The antimicrobial tests were confirmed with non-culture-based techniques namely bioluminescent imaging and LIVE/DEAD Baclight to determine the antimicrobial efficiency against viable but non-culturable (VNBC) cells. This

was done to quantify the cells that enter a dormant state during contact with the antimicrobial fibres and to eliminate the chances of overestimating the antimicrobial efficiency of the nanofibers.

Opsomming

Water is 'n onmiskenbare regte vir alle mense en hul welstand is afhanklik van gelyke toegang tot 'n veilige, geskikte en bekostigbare watervoorsiening. Die wêreld in die gesig staar formidabele uitdagings in die vergadering stygende eise van skoon water as die beskikbare voorraad van vars- water verminder weens (i) langdurige droogtes, (ii) bevolkingsgroei, (iii) strenger gesondheidsverwante gebaseer regulasies, en (iv) mededingende eise van 'n verskeidenheid van gebruikers.

Die doel van hierdie studie is om voor te berei en te vergelyk drie tipes van poli (etileen mede -vinyl alkohol) (EVOH) nanovesels en bepaal watter tipe vesel meeste geskik is vir die toepassing in water sanitasie sal wees. Toetsing van antimikrobiese aktiwiteit van die AquaQure bevat EVOH nanovesels, sowel as loging uit metaalione wat toksisiteit en herbruikbaarheid van die EVOH nanovesels verander word met die AquaQure biologiese middels kon impliseer sal ondersoek word.

As 'n poging om weg te doen met die konvensionele metodes van watersuiwering dat die gebruik van chemikalieë wat skadelike stowwe bekend stel aan die omgewing, vervaardiging en karakterisering van anti- mikrobiese polimeer nanovesels met 'n nanobiocide sal gedoen word in hierdie studie betrek. Tweedens sal die probleem van bio op filtrasie membrane aangespreek word deur die vervaardiging en toets polimeer nanovesels met verskillende grade van hidrolise.

Om water sanitasie, drie tipes EVOH nanovesels, spreek (i) 27mol% (ii) 32mol% en (iii) 44mol% etileen inhoud om voorbereid te wees met behulp van enkele naald elektrospin. AquaQure 'n waterige antimikrobiese agent wat hoofsaaklik Cu^{2+} en Zn^{2+} -ione sal gebruik word as 'n toevoeging tot die polimeer oplossings.

Die nanofibrous matte, netjiese EVOH en verander EVOH / AquaQure is suksesvol vervaardig met behulp van die konvensionele enkele naald elektrospin. Die inlywing van die biologiese middels is bevestig deur EDX. ATR-FTIR, TGA, is DSC en SEM tegnieke wat gebruik word om chemiese en termiese analise van die nanovesels doen in vergelyking met netjiese EVOH nanovesels. Die hydrophobicity van nanofibrous matte is gemeet deur statiese kontak hoek metings.

Die toets van antimikrobiese aktiwiteit van die AquaQure bevat EVOH nanovesels teen die gram positiewe en Gram negatiewe Bioluminescent stamme van *Staphylococcus aureus* Xen-

36 en *Escherichia coli* Xen-14 onderskeidelik. Die antimikrobiese toetse bevestig met nie-kultuur gebaseer tegnieke naamlik Bioluminescent beelding en leef / dooie Baclight om die antimikrobiese doeltreffendheid teen lewensvatbare maar nie-culturable (VNBC) selle te bepaal. Dit is gedoen om die selle wat 'n dormante staat voer tydens kontak met die antimikrobiese vesels kwantifiseer en om die kanse van die oorskatting antimikrobiese doeltreffendheid van die nanovesels te skakel.

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In all my achievements and what is yet to come I offer gratitude to the MOST HIGH who gives me strength to achieve all things…

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List of Abbreviations

AqQ	AquaQure
ATR-FTIR	Attenuated total reflectance-Fourier transform infra-red spectroscopy
BHI	Brain Heart Infusion
BLI	Bioluminescence imaging
CFU	Colony forming units
Cu	Copper
DMSO	Dimethylsulfooxide
DNA	Deoxyribonucleic acid
DSC	Differential scanning Calorimetry
EDX	Energy dispersive X-ray Spectroscopy
ENMs	Electrospun nanofibrous membranes
EVA	Ethylene-vinyl acetate
EVOH	Poly (ethylene-co-vinyl alcohol)
FO	Forward osmosis
FTIR	Fourier transform infra-red spectroscopy
GRAS	Generally recognised as safe
ICP-AES	Inductive Coupled Plasma-Atomic Emission Spectroscopy
IVIS	in vivo imaging system
MF	Microfiltration
NCEZID	Centers for Disease Control and Prevention National Center for Emerging and Zoonotic Infectious Diseases
NF	Nanofiltration
NPs	Nanoparticles
OECD	Organisation for Economic Co-operation and Development
PEO	Poly (ethylene oxide)
PRO	Pressure retarded osmosis
SEM	Scanning Electron Microscopy
Tc	Crystallization temperature
Tg	Glass transition temperature
TGA	Thermogravimetric analysis
Tm	Melting temperature

UF	Ultrafiltration
USA	United States of America
UV	Ultra violet
UV/Vis	Ultra violet-visible
VNBC	Viable but non-culturable
VO	Vinyl alcohol
WHO	World Health Organisation
Wt. %	Weight percent
Xc	Crystallinity
Zn	Zinc

Chapter 1

Introduction and objectives

1.1 Introduction

Access to a safe, adequate and affordable supply of water is essential for the health and well-being of all people. In addition, this access should be an undeniable right for all (Douglas et al., 2006). The world is presently facing some serious challenges in meeting the ever rising demands for clean potable water. The availability of fresh water is decreasing due to (i) extended droughts, (ii) a growing population, (iii) much more regulations regarding health and safety and (iv) increasing competition from various users (US Bureau of Reclamation, 2003).

WHO (2004) reported that more than one out of six people lack access to safe drinking water, and more than two out of six people lack adequate sanitation. Infectious water borne diseases are the primary killer of children under five years. According to Ross (Ross, 2008) more people die annually from exposure to unsafe water than from violence including war.

Nanotechnology is becoming more important for use in water systems. Nanotechnology is reported as being potentially important for three key purposes: treatment and remediation, sensing and detection, and pollution prevention (OECD, 2011.). Established techniques for water treatment can have drawbacks which nanotechnology may help to address because of its cost effectiveness and environmentally acceptable water purification processes (Savage and Diallo, 2005).

Recently reported research results advances indicate that many of the present issues involving water quality could be at least partially resolved using nanostructured materials (nanoparticles, nanofiltration and similar products) (Savage and Diallo, 2005). Here a review by Van der Bruggen and Vandercasteele (2003) on the use of nanofiltration to remove contaminants including naturally occurring organic matter, biological contaminants, organic pollutants, and dissolved inorganic salts and toxins found in ground and surface water. Biofilms are a concern especially in drinking water systems because they are a source of contamination (Momba et al., 2000), which has a major impact on the biological stability, hygienic safety (Emtiazi et al., 2004) and the overall water quality (Khiari and Watson, 2007; Ludwig et al., 2007).

Biofilms are complex communities of surface-attached microorganisms, comprised either of single or multiple species (Costerton, 1995). They can form in almost any hydrated environment that has the proper nutrient conditions and can cause fouling. In most industrial

and clinical processes it has been found out that biofouling can be difficult to control and has several severe implications (Mikkelsen and Keiding, 2002; Sponza, 2002).

Smith (2006) believes that nanotechnology for water remediation will help in the world's water security and consequently in food security. There are concerns that arise from the same properties i.e. size, shape, reactivity, conductivity that make nanoparticles so useful to mankind and can consequently make them potentially harmful to the environment and toxic to humans. These concerns result from lack of understanding of the fate and behaviour of nanoparticles in humans and the environment but presently how this will affect their toxicity in the long term is unclear. Nanofibres, nanobiocides and nanofiltration are forms of nanotechnology that are currently being used in water treatment (Nanotechnology and Water).

A widely used method to inactivate pathogenic micro-organisms in water and wastewater and for preventing waterborne infectious diseases throughout the world is the use of oxidation biocides such as chlorination (Crittenden, 2005). However, some studies have reported that the effectiveness of the process is reduced by turbidity, suspended solids and the presence of nitrogen compounds such as ammonia and nitrite (Lazarova et al., 1999). The use of chlorine in water treatment gives rise to undesirable by-products suspected to pose a hazard to humans and the environment (Minear and Amy, 1996), but also the rise in resistant pathogens is considered as being very problematic.

Nanofibers are one dimensional materials that have high specific surface area because of their small diameters. Nanofiber membranes are known to be highly porous with superior pore interconnectivity. The combination of membrane porosity with the properties from the polymers themselves impart nanofibrous membranes with a lot of appealing properties for advanced applications (Fang et al., 2003). Therefore functionalised nanofibers with a biocide can be used as a cost-effective alternative for chlorine.

Despite several methods that have been developed for the production of nanofibers, electrospinning is regarded as the most promising technique to produce continuous nanofibers in a non-woven form on a large scale and the fibre diameter can be adjusted from nanometres to micrometers (Li and Xia, 2004). Ways in which nanofibers can be fabricated include template (Ikegame et al., 2003), self-assembly (Hong et al., 2003), phase separation (Ma and Zhang, 1999), melt- blowing (Ellison et al., 2007) and electrospinning (Doshi and Reneker, 1995; Lin et al., 2004; Lin et al., 2005a; Fang et al., 2007; Xue et al., 2009; Fang et al., 2010).

Borkow and Gabbay (2005) reported that metal ions either alone or in complexes, have been used to disinfect fluids, solids and tissues for centuries. Therefore anti-microbial nanofibers can be synthesized by surface modification and blending of polymers with metal ions. Antimicrobial properties of copper and zinc have been reported (Sheikh et al. 2011; Grace et al., 2007).

A number of studies have been conducted by Sheikh et al., for example which have tried to explain how copper and zinc disrupt the bacterial cells. These studies have led to the preposition that states that the ions bind to the sulfhydryl-groups of respiratory enzymes in the cell membrane (PoolRx Worldwide Inc., 2012). Other theories suggest that the ions cause (i) the potassium that is inside the bacterial cells to leak out via the outermost membrane, (ii) an imbalance in the osmotic pressure, (iii) binding to proteins that do not require copper or zinc and (iv) the production of peroxides which in turn cause oxidative stress (Sheikh et al., 2011).

The aim of this study is to prepare and compare three types of poly (ethylene-co-vinyl alcohol) EVOH nanofibers and determine which type of fibre will be most suitable for the application in water sanitation. Determination of antimicrobial activity of the AquaQure containing EVOH nanofibers, as well as release studies of metal ions which could imply toxicity and reusability of the EVOH nanofibers modified with the AquaQure biocide will be investigated.

As an effort to do away with the conventional methods of water purification that involve the use of chemicals which introduce harmful substances into the environment, fabrication and characterization of anti-microbial polymer nanofibers with a nanobiocide will be done in this study. In order to determine the best suited membrane for the intended application, three grades of poly (ethylene-co-vinyl alcohol) will be fabricated and tested. Single needle electrospinning will be employed to fabricate the nanofibers. The chosen grades of EVOH are (i) 27 mol. % (ii) 32 mol. % and (iii) 44 mol. % ethylene content. An aqueous solution of metal cations commercially known as AquaQure will be used as the antimicrobial agent. This biocide mainly contains Cu^{2+} and Zn^{2+} ions.

EVOH is a hydrophilic, semi crystalline polymer commonly used because of its good chemical resistance, good thermal stability, good physical properties, excellent biocompatibility, and low cost. Moreover, this polymer is generally recognized as safe (GRAS) (Lopez-Rubio et al., 2009). It is derived from the hydrolysis of poly (ethylene-co-vinyl acetate) but there is always a small residual amount of acetate groups from incomplete hydrolysis. The material is

commercially available in a range of compositions, most commonly with vinyl alcohol contents of about 55–70 mole % (Kenawy et al., 2003).

Furthermore, to determine which membrane would be the most suitable, the objective is to exploit their different degrees of hydrolysis which renders them different properties. The nature of the membrane is important because it determines how it will interact with the biocide and in turn interact with the contaminants in the dirty water. EVOH was chosen because of its hydrophilic nature and it has been reported that membranes with hydrophilic surfaces are less sensitive to fouling than hydrophobic membranes (Knoell et al., 1999). It is reported that generally bacteria with hydrophobic properties prefer hydrophobic material surfaces and the opposite is true (An and Friedman, 1998).

Bacterial attachment at the membrane surface causes initial biofilm and this can be avoided by the use of a hydrophilic membrane surface. Most of the hydrophilic ultrafiltration membranes have fixed negative charges on their surfaces and this in turn prevents the negatively charged colloidal particles to settle on the membrane surface, and therefore slows down the membrane fouling process (Düputell and Staude, 1993).

Khulbe et al. (2009) reported that surface properties of polymers are of great importance in many sectors of industrial applications such as the separation of gasses, liquid mixtures, bonding, coating, adhesion and so forth. Their performances depend on the properties of their surfaces, since membranes may be considered as one of the surface phenomena. Surface contamination which may lead to deterioration in membrane performance is also known to be dependent on the membrane surface properties.

1.2 Aim and objectives

The aim of this study is to prepare and compare three types of poly (ethylene-co-vinyl alcohol) nanofibers and determine which type of fibre will be most suitable for the application in water sanitation. A nanobiocide will be added to the polymer solutions to enhance their antimicrobial properties.

Under this aim the specific objective is to investigate if the hydrophobicity of the nanofibers affects the properties of the material in water sanitation applications.

The work is divided into three main stages;

- Fabrication and characterisation of nanofibrous mats

- Antibacterial tests
- Release studies

1.3 Structure of thesis

1.3.1 Chapter 1

This chapter has an introduction plus the aim and objectives of this study.

1.3.2 Chapter 2

This is the literature review of all the previous work done on the topic of nanotechnology and water sanitation.

1.3.3 Chapter 3

This chapter focuses on the first experimental part of the study which is the synthesis and characterisation of the nanofibers.

1.3.4 Chapter 4

All the antibacterial tests and results including zone of inhibition and release studies experimental work is presented in this chapter.

1.3.5 Chapter 5

This chapter presents the conclusion for the study.

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Chapter 2

Historical background and literature review

2.1 Introduction

The great importance of water for man makes the accurate management of this natural resource a priority in order to preserve its sustainability. Access to safe drinking water is a fundamental human need and therefore, a basic right. Water shortage is increasingly recognized as one of the most immediate and serious environmental threats to humankind. The lack of clean, fresh water is a major cause of well-known problems worldwide. Many people do not have access to safe drinking water, have little or no sanitation and many deaths are recorded annually from diseases transmitted through unsafe water or human excreta (Shannon *et al.*, 2008).

Inadequate water management is speeding up the depletion of surface and groundwater resources. Water quality has deteriorated drastically due to domestic and industrial pollution sources as well as nonpoint sources. Wastewater contains pathogens (viruses, bacteria, protozoa, and helminths) and chemical constituents that are of concern if the wastewater is to be used beneficially.

The human wellbeing impacts of waterborne transmission differ in severity from mellow gastroenteritis to severe and at times fatal diarrhoea, dysentery, hepatitis and typhoid fever. The gastrointestinal tract is the most infected by common bacterial pathogens that are transmitted by water and find themselves in the environment through faeces from infected humans and animals. Nevertheless, there are also some waterborne bacterial pathogens, such as *Legionella, Burkholderia pseudomallei* and atypical mycobacteria that can grow in water and soil.

As a way to try and solve the water scarcity problem, new and sustainable methods to enhance supplies and decontaminate water can be developed and put to use in order to serve people worldwide (Montgomery and Elimelech, 2007). Ongoing research being pursued is focused on how to improve the current water treatment methods that include disinfection, decontamination, re-use and desalination methods to work hand in hand to improve health, protect the environment, and reduce water scarcity, not just in the industrialized world but as well as in the developing world, where less chemical and energy intensive technologies are greatly needed.

A widely used method to inactivate pathogenic microorganisms in water and wastewater and for preventing waterborne diseases throughout the world is the application of membrane technologies; ozonisation, chlorination, and UV light (Lev et al., 2011). Membrane separation technology has increasingly been used in water and wastewater treatment today because the membrane filtration processes are relatively fast, efficient and practical (Van der Bruggen and Vandecasteele, 2002). Membrane separation technology offers great promise to meet the more stringent regulatory requirements for water quality that cannot be easily met by conventional treatment technologies. Membrane separation technology has also made alternative water supply from non- traditional sources, such as seawater desalination and wastewater reclamation, possible solutions to address the growing global scarcity of traditional water sources (Liu et al., 2010).

In the wake of microorganisms becoming unsusceptible to numerous antimicrobial agents, there is increased demand for improved disinfection methods which include the use of metal ions (Ruparelia *et al.*, 2008). Metal ions are known biocides, this has been known and applied for centuries, especially the antimicrobial activity of metals such as silver (Ag), copper (Cu), gold (Au), titanium (Ti), and zinc (Zn), each having various properties, potencies and spectra of activity (Adibkia et al., 2014). The use of silver and copper ions as superior disinfectants for wastewater generated from hospitals containing infectious microorganisms has been recommended (Lin et al., 1996). Though the leftover copper and silver ions in the treated water may have negative effects on the human health (Blanc et al., 2005).

Historical records suggest that the importance of pure water was emphasized even during ancient civilizations. Ancient civilizations started the use of aqueducts for creating efficient water transport networks (Indus valley, Greek, Roman and American civilizations). Hippocrates, the father of medicine, linked the importance of water to overall well-being of the human health. In early 1600s, Sir Francis Bacon scientifically tested the idea of a sand filter for desalination in 1627 (Pradeep, 2009). During the course of over 150 years, understanding of water quality, its effects on health and methods for water purification has undergone a sea-change.

2.2 Electrospinning

In 1897 Rayleigh first discovered electrospinning but Zeleny studied it further in 1914 with respect to the electrospray technique (Zeleny, 1914). The fibre spinning technique was developed by Formals in 1934 from which he managed to produce artificial filaments making

use of electrical charges. Formals was able to patent his experimental setup in the late 1930s (Formhals, 1934). Various contributions from scientists that include Taylor, Saville and Denn towards electrically induced jets laid the foundation for the electrospinning research that occurred in the late 1960s and early 1970s (Taylor, 1969; Saville, 1970; Denn, 1975). With the rapid development of nanotechnology, the electrospinning process has developed a great deal with time.

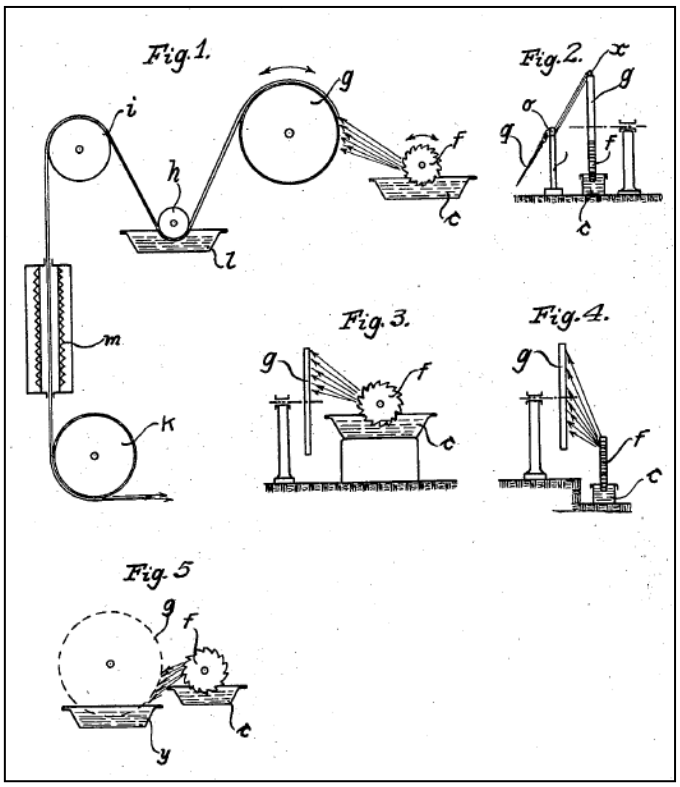

Figure 2.1: Formhals patent images, process and apparatus for preparing artificial threads (Formals, 1934).

2.2.1 Principle of electrospinning

Electrospinning can be described as a unique approach that makes use of electrostatic forces to produce fine fibres from polymer solutions or melts. The resultant fibres have a small diameter that ranges from nanometre to micrometre and have a larger surface area compared to the ones obtained from conventional spinning processes (Bhardwaj, 2010).

The principle of electrospinning is similar to that of electrospraying. A charge is induced by an electric field to the polymer solution or melt that is being held by its surface tension at the end of a capillary tube or needle. Charge repulsions that occur cause a force directly opposite to the

surface tension. As the intensity of the electric field is increased, the hemispherical surface of the solution at the tip of the capillary tube elongates to form a conical shape known as the Taylor cone (Kozhenkov and Fuks, 1976).

There is a critical value at which the repulsive electrical forces overcome the surface tension forces that needs to be reached in order for a charged jet of the solution to be ejected from the tip of the Taylor cone. The trajectory of the polymer jet is susceptible to any electric field because it is charged. As the jet travels in air, the solvent evaporates, leaving behind a charged polymer fibre. Continuous fibres are collected in the form of a non-woven fabric (Doshi and Reneker, 1995).

There are a number of processing techniques that can be used to produce polymeric nanofibers besides electrospinning such as drawing, template synthesis, phase separation and self-assembly (Angammana, 2011). Electrospinning is the one mostly used technique because it is a straightforward and inexpensive process that produces continuous nanofibers from submicron diameters down to nanometre diameters. Also it is clearly advantageous since it can be manipulated in a variety of ways depending on the desired application (Franco et al., 2012)

Adjustment of solution concentration, injection rate, supplied voltage and collecting distance has shown to have an effect on the resulting fibre morphology. When these production techniques are compared, electrospinning proves to have more advantages over the others as shown in the table below. Therefore the electrospinning process can thus be considered the only method that can be further developed for the mass production of continuous nanofibers from a variety of polymers (Ramakrishna, 2005).

2.2.2 Electrospinning setup

Today, the most basic setup involves a nozzle, high voltage supply (between 0-30 kV) and a grounded collector, diagrammed in Figure 2.1.

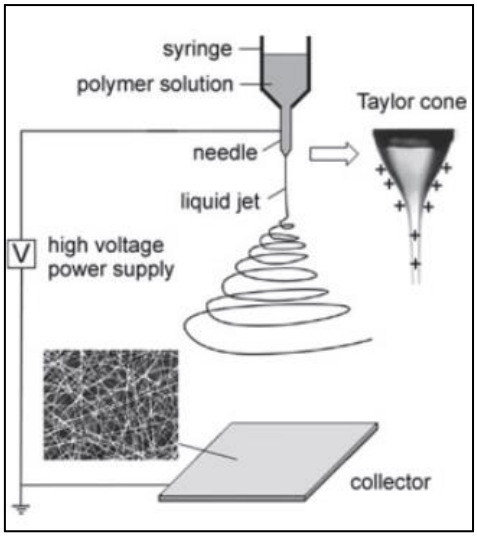

Figure 2.2: Electrospinning setup (Castillo, 2012).

To optimise the electrospinning process, the experimental setup can be modified for instance in this study, a pump is added to ensure a constant flow rate through a syringe, which is used as the nozzle. Different collector plate designs have been added as a way to optimise as well as to overcome the shortcomings of conventional electrospinning schemes (Ramakrishna et al., 2006).

2.2.3 Electrospinning mechanism

The solution jet is subjected to forces and instabilities which can reinforce influence or even compete with each other (Mariën, 2011). There are four major regions that can be observed during electrospinning. These are the Taylor cone, steady jet, bending instability and collection regions (Pham et al., 2006; Reneker and Chun, 1996).

Taylor cone region

According to Taylor, jet initiation is when the Taylor cone is formed and the intensity of the electric field reaches a critical value and the surface tension is overcome and the liquid jet is forced from the tip of the cone, where the highest charge density is located (Castillo, 2012). The solvent evaporates during a whipping process where the stretched polymer fibre is randomly collected on a grounded metal plate of opposite polarity than the charged jet.

Steady jet region

In the steady jet region is when the amount of polymer pumped through the nozzle equals the deposited amount of polymer as nanofibers and when a stable Taylor cone with time is obtained

(Goethals, 2010; De Vrieze, 2010). As a result, nanofibers are electrospun without a change in properties and the process is stable, thus sputtering, clogging or degradation can be prevented. Another important consequence is the possibility to upscale the electrospinning processes, where the preservation of properties should be guaranteed.

Bending instability region

The start of bending instability can be observed in the bending instability region. The bending takes a complex path, and other changes in the shape also occur, as can be seen in Figure 2.3 below.

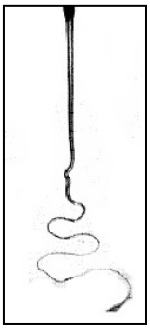

Figure 2.3: Bending instability of polymer jet (Taylor, 1969).

A lot of research has been done trying to model the electrospinning mechanism mathematically and this is known as jet modelling. The jet modelling mechanism generally takes place in three stages known as initiation, thinning and solidification of the jet (Gule, 2011). There are two main jet instabilities, non-electrically induced and electrically induced instabilities. There is the Rayleigh instability which is non-electrically induced instability and driven by the surface tension. By breaking up the jet, the surface area and thus thermodynamic surface energy is minimized (Greiner and Wendorff, 2007). It can be inhibited at high electrostatic fields when the surface tension will not be affecting the jet anymore.

The two electrically induced instabilities are axisymmetrical instability and the non-axisymmetric or bending instability. The axisymmetrical instability occurs when a variation in surface charges on the jet occurs in a complex mechanism of coupled forces and beads can be formed (De Schoenmaker, 2008). These beads weaken the fibres. The non-axisymmetric instability dominates when at higher surface charges, a situation which normally occurs in an electrospinning process. This type of instability varies the bending or whipping movement of the jet affecting the elongation and thinning of the fibres (Shin, 2001).

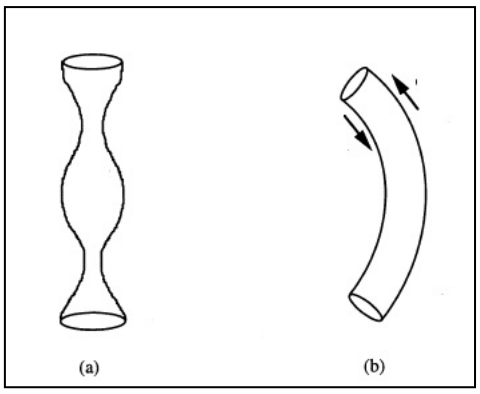

Figure 2.4 : (a) Axisymmetrical instability and (b) Bending instability (Mariën, 2011).

The mechanism of electrospinning can be affected by solution and process parameters and ambient conditions (Mariën, 2011). These parameters described in section 2.2.4 can affect greatly the morphology of the electrospun fibres.

Collection region

The polymer jet stops at the collection region. A number of ways are employed to collect the polymer fibres that remain after the solvent evaporates such as metal screens (aluminium foil paper), water or any other appropriate liquids for those polymers dissolved in non-volatile solvents and also aerodynamic currents or mechanical reels (Reneker and Chun, 1996). If the jet arrives with a high velocity at a stationary collector, the jet tends to coil or fold. Since the jet is charged, a fibre lying on the collector tends to repel fibres that arrive later.

2.2.4 Parameters that affect fibre morphology

The spinning process depends on various parameters that are discussed below and the morphology and diameter of electrospun fibers can be controlled by controlling these parameters.

Solution parameters

Among the solution parameters, the most important are the polymer or solution concentration, molecular weight and conductivity. Concentration and molecular weight indirectly affect the viscosity of the solution which is a significant parameter that influences the diameter and morphology of the fibre (Angammana, 2011). Solution concentration affects fibre diameter and is crucial to successful fibre collection. Solution concentration that is too low or high results in an unstable jet (Zong, 2002). Studies show a strong correlation to fibre diameter (Erman et al.,

2002). For concentrated solutions, a second population of small fibres appears in addition to the normal fibres (Deitzel, 2001).

The conductivity of a polymer solution contributes to the elongation level of the jet. A significant reduction in the diameter of the electrospun nanofibers can therefore be observed when the electrical conductivity of the solution is increased because the jet carries more charges. By adding a salt or a polyelectrolyte to the electrospinning solution, the electrical forces of the increased charge carried by the electrospinning jet cause the jet to elongate, and uniform fibres are produced thus no beaded fibres a produced. However, if the conductivity of the solution is too high, this can causes difficulties in electrospinning the fibres even if very high voltages are employed. Similarly, it is difficult to form fibres if the solution conductivity is low (Supaphol et al., 2005).

Process parameters

One of the major parameters that is used to alter the electric field is the voltage applied between the two electrodes i.e. the needle and the collector plate. For the fibre diameter purposes however, the applied voltage will result in a smaller fibre diameter if increased, but only to a certain point, where the jet diameter begins to increase again due to the increased repulsion force between charges, resulting into a higher mass flow (Subbiah, 2005). Thus there is an ideal applied voltage that results in a minimum fibre diameter depending on the polymer and interacting parameters (Baumgarten, 1971).

Varying the tip to collector distance causes a change in the behaviour of the electrospun jet and the morphology of the resultant nanofibers. The desired nanofibers can be collected if a reasonable time is allowed for the evaporation of most of the solvents. Though a larger gap distance results in a weaker electric field, theoretically the larger flight distance should result in a fully evaporated solution but when the distance between the needle tip and the collector plate is decreased, the resultant fibres may fuse to become an interconnected fibre mesh due to the presence of excess solvents (Angamanna, 2011;Castillo, 2012).

Ambient conditions

The interaction between the surrounding environment and the electrospinning jet may affect the electrospinning process and fibre morphology. The relative humidity of the electrospinning chamber can affect the time it takes for the jet to solidify due to water absorbed by the solution, and thereby cause changes in the morphology of the fibres (De Vrieze et al., 2009).

Temperature also affects the evaporation of the solvent. The lower the temperature, the slower the rate of evaporation.

2.3 Polymer nanofibers: properties and applications

Electrospinning provides a straightforward way to produce long polymer fibres. Research has shown that the smallest polymer nanofiber must contain one polymer molecule. It is known that a single polymer molecule has a diameter of a few tenths of a nanometre (Reneker and Chun, 1996). Nanofibers are the link between the nanoscale world and the macroscale world, since the diameters are in the nanometre range and the lengths are kilometres. Many synthetic and natural polymers as well as polymer blends have recently been electrospun to form nanofibers for a variety of applications. Bringing materials to the nanometre scale not only improves their properties, but also affords it new advanced characteristics beyond bulk materials (Wang et. al., 2009).

Nanofibers are a type of one-dimensional nanostructure, with diameters varying from 1 nm to 1000 nm. Their nanometre diameter provides polymer nanofibers with several outstanding characteristics, such as a very large surface area to volume ratio (aspect ratio). For a nanofiber, this ratio can be as large as 10^3 times of that of a microfiber (Angammana, 2011). Other advantages include flexibility in surface functionality, and superior mechanical performance compared with any other existing form of the material (Huang et al., 2003). These excellent properties make polymer nanofibers optimal candidates for many important applications and offer the potential for significant improvements in current technology and the development of applications in new areas. As can be seen in the Figure 2.5.

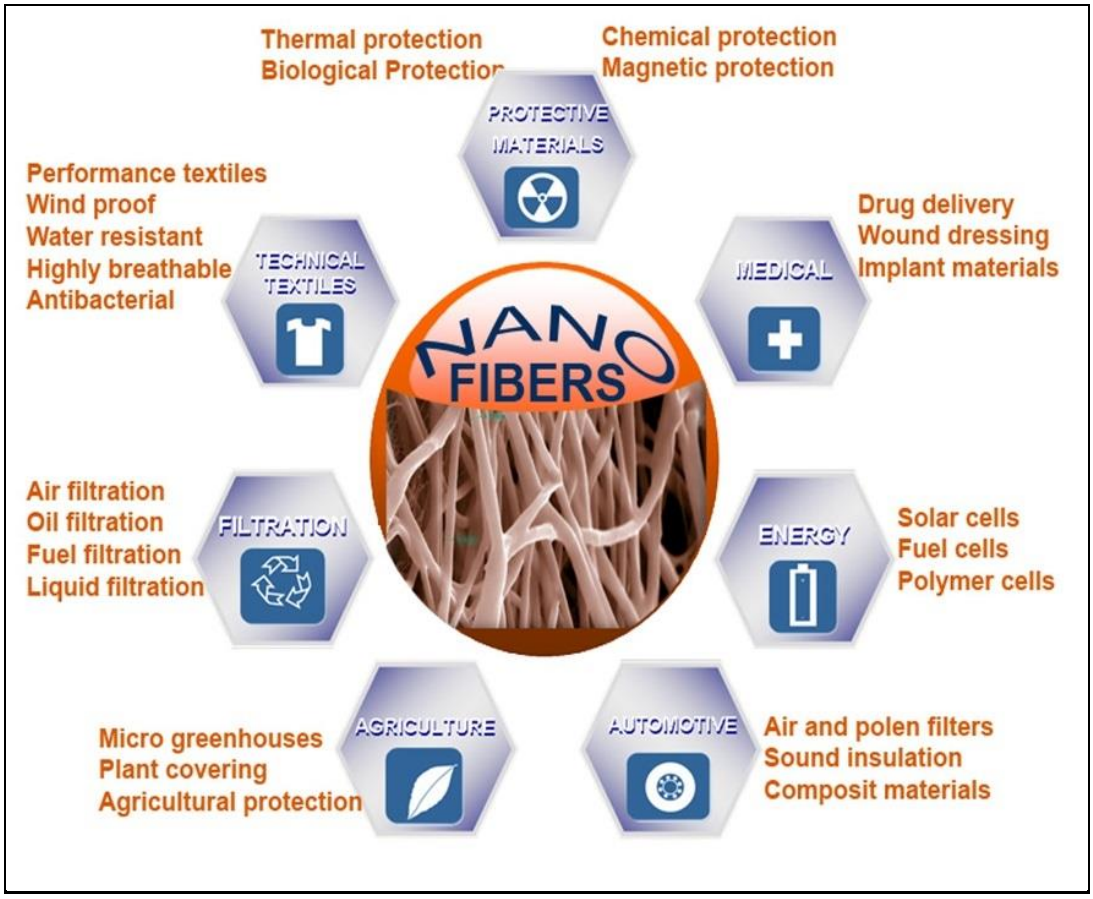

Figure 2.5: Nanofiber Applications in Industry (AzoNano, 2017).

Quite a number of polymer matrices are made from non-renewable and non-biodegradable materials rendering them unsuitable for environmental applications.

2.4 Modification of polymer nanofibers

The nanofibers prepared by electrospinning can be modified through a number of ways to improve their properties and or to increase the diversity of materials that could be processed as fibrous nanostructures (Li and Xia, 2004). In order for the electrospun nanofibers to meet up with their wide range of applications, some kind of modification has to be done. There are several ways in which this can be done, resulting in nanofibers with enhanced properties. Modification processes can be divided into two main groups, surface modification and bulk modification.

The surface properties of nanofibers are of importance in the applications such as biomaterials, filtration and electronics. Though the electrospinning technique could produce nanofibers with special structures and morphologies, it is difficult to prepare the fibres with desirable surface

properties meet some applications such as the surface features affect wet-ability, adsorption and adhesion of the fibres.

Surface modifications of the electrospun nanofibers enhance the nanofibers matrix properties such as availability of functional groups to combat microbial colonization and prevent cross contamination in medical sectors (Page et al., 2009; Banerjee et al., 2011).

Surface modification can be done by inducing some polymerization reactions on the fibre surface, adsorption or immobilization of special active agents (such as drugs, some proteins) onto the fibre surface, surface coating (Helsa-automotive, 2011) where a second functional polymer is coated onto the bulk polymer surface (Mariën, 2011), solvent vapour treatment (Finetex Technology, 2011), grafting or blending (Mariën, 2011) and plasma treatment (US Global Nanospace, 2011) which is used to create a surface change rather than depositing functional polymers (Mariën, 2011).

Bulk modification is whereby the polymer solution is functionalized, resulting in altered nanofiber properties. The most used techniques are dispersed electrospinning, sol-gel method and coaxial electrospinning (Sundarrajan and Ramakrishna, 2010). Some of these methods can enhance electrospinning, such as increasing the solubility by using another solvent or blend a polymer with a polymer that can be electrospun (Li and Xia, 2004).

2.5 Biocides

Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth, without being in general toxic to surrounding tissue. Biocides are chemical agents used to kill harmful microorganisms in order to preserve health and to protect product integrity and the effectiveness of biocides varies with concentration and duration of exposure (Chattopadhyay et al., 2004). They are a common part of everyday life and they are also known as antimicrobials, pesticides or algaecides. Biocides are used for drinking water treatment, wastewater treatment, ship ballast water treatment, disinfectants and as antifouling agents that prevent molluscs from accumulating in industrial pipes. In the water treatment field, a biocide is a substance that inhibits the growth of nuisance organisms such as algae, bacteria and fungi. Biocides are produced in liquid and powder forms, in ready-to-use formulations or as concentrates, and are applied using a variety of techniques.

2.5.1 Nanobiocides

The functional activities of nanoparticles are influenced largely by the particle size. Therefore, nanoparticles have received great attention due to their unique physical, chemical, and effective biological properties in various fields. The properties of nanoparticles can easily be altered by reducing or changing their size, especially when the manipulations are done at the nanometre scale (Seil and Webster, 2012).

Nanobiocides are antimicrobial nanoparticles which fall into two categories of metals (silver, gold, copper and zinc) and metal oxides (titanium oxide). Synthetic nanoparticles such as fullerenes and naturally occurring antimicrobial materials such as chitosan (Cloete and Botes, 2010) are regarded as nanobiocides. Metal ions, either alone or in complexes, have been used to disinfect fluids, solids and tissues for centuries (Block, 2001; Dollwet and Sorenson, 2001). Nanoparticles are used as detectors and removers of poisonous contaminants such as heavy metals, pesticides, halogenated organics and microorganisms from drinking water during water treatment (Gule, 2011).

Albright and Wilson, 2001 found that sensitivity in descending order to heavy metals of microflora in water was Ag, Cu, Ni, Ba, Cr, Hg, Zn, Na, Cd (Bokow and Gabbay, 2005). In this study the biocide used is mainly composed of copper and zinc ions.

2.5.2 Copper and Zinc as nanobiocides

Copper just like conventional silver has antibacterial properties. Copper, both in its metallic and ionic forms, has been exploited since ancient times for medical uses in countless cultures around the globe (Bokow et al., 2004). Copper metal's toxicity is being owed to the fact that it tends to alternate between its copper (I) and copper (II) oxidation states. Copper attacks the respiratory enzymes in bacteria, presumably by binding to groups containing; sulfhydryl, amine, and carboxyl moieties. Copper is also believed to facilitate hydrolysis or nucleophilic displacement reactions in peptide chains or nucleic acids. Copper has chelating capabilities therefore is able to chelate with phosphate groups resulting in the opening of the DNA double helices (PoolRx, 2012).

Söderberg et al., 1990, have shown that zinc naturally reduces the activity of a wide range of (mostly Gram-positive) bacteria strains. The health benefits of zinc in people are multi factorial and are based on (1) direct antiviral effects of zinc ions, (2) amplification and maintenance of immunity, (3) augmentation of interferon activity and (4) a natural defence mechanism at the cell membrane level. However, the specific mode of action of zinc in vivo is unclear.

There are several ways in which zinc is believed to exert its therapeutic effect. These include production inhibition of the viral capsid protein, enabling the production of gamma interferon, and stabilizing and protecting plasma membranes against lysis by cytotoxic agents. Zinc is believed to also inhibit the rhinoviral interaction with intercellular adhesion molecules, this is the site where the virus initially binds to epithelial cells. The release of histamine and other inflammatory mediators from mast cell granules may be interrupted by zinc (PoolRx, 2012). Figure 2.5 shows how metal ions cause damage to the bacterial cells.

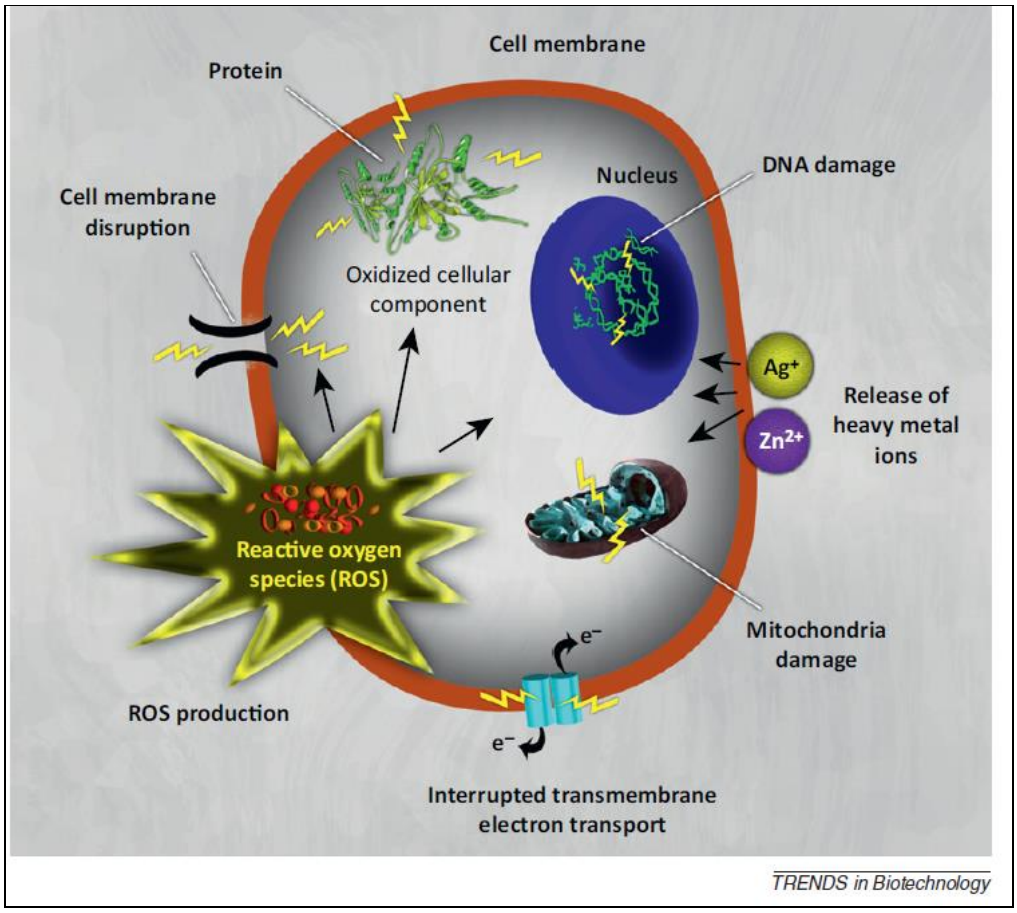

Figure 2.6: Mechanisms of nanoparticles (NPs) against bacteria (Trends In Biotechnology, 2012).

2.6 Bacteria used in the study

Common waterborne pathogens are introduced into drinking water supplies in human or animal faeces. Contaminated water can be the source of large outbreaks of disease, such as cholera and dysentery. To quantify potential pathogen loads in water resources, indicator organisms are monitored. Escherichia coli for example has been used for decades as an indicator organism to assess levels in rivers, lakes, estuaries, and coastal waters (Pandey *et al.*, 2014).

Properties of bacteria, and thus the way to destroy or inactivate them, are highly specific to the respective bacterial strains. The bacterial cell wall is designed to provide strength, rigidity, and shape, and to protect the cell from osmotic rupture and mechanical damage (Singleton et al., 2004). According to their structure, components, and functions, the bacteria cell wall can be divided into two main categories i.e. Gram positive (+) and Gram negative (–) bacteria. Gram-negative bacteria's cell wall comprises of a thin peptidoglycan layer and contains an outer membrane, which covers the surface membrane. This is a more complex cell wall both structurally and chemically in comparison to the cell wall of the Gram positive.

On the other hand, the Gram-positive (e.g. *Staphylococcus aureus*) the peptidoglycan has a layer (i.e., 20–50 nm) of which is attached to teichoic acids that are unique to the Gram-positive cell wall. Figure 2.7 shows the differences in the cell walls.

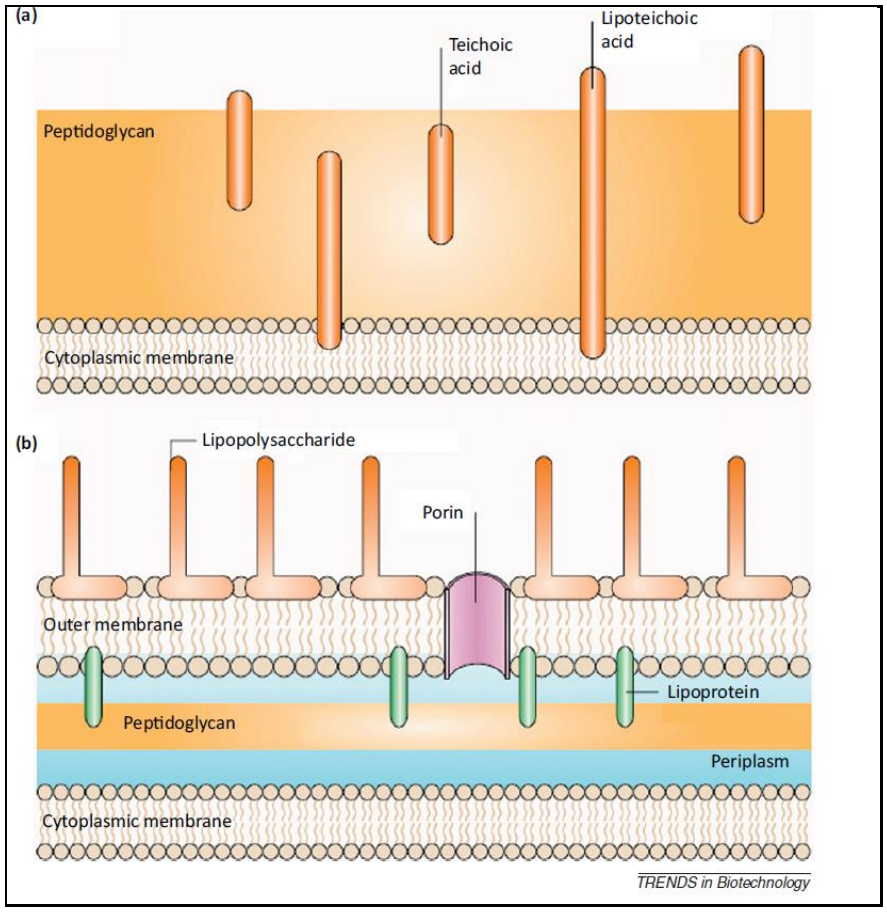

Figure 2.7: Differences in the (a) Gram positive and (b) Gram negative cell walls. (Cabeen and Jacobs-Wagner, 2005)

2.6.1 Escherichia coli (E.coli)

Escherichia coli are non-spore-forming, gram-negative bacteria, usually motile by peritrichous flagella. This bacteria is found in the environment, food, and intestines of both people and animals. *E. coli* are a large and diverse group of bacteria and form rod-shaped cells 2.0 – 6.0 µm in length and 1.1–1.5 µm in width, though the cells may vary from coccal to long filamentous rods (Wilson and Miles, 1964). Even though most strains are known to be harmless, others can cause illness. The less commonly encountered *E. coli* strains found within the environment and in potable water systems are very much capable of giving rise to diseases usually in the form of diarrhoea. The way in which *E. coli* causes diarrhoea differs between strains.

It is reported that the other strains of *E. coli* that do not cause diarrhoea are the most common cause of acute urinary tract infections as well as urinary tract sepsis. It has also been known to cause neonatal meningitis and sepsis and also abscesses in a number of organ systems while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (NCEZID, 2017).

E. coli is known to have a reservoir in the intestines of human beings and other warm blooded animals. It is released into the environment through faecal matter. The bacteria is known to survive in the environment but is not able to reproduce. The routes of exposure and transmission in humans are faecal to oral, therefore through food, water, and person to person. Water related outbreaks are directly linked to water contaminated with sewage. Risk from drinking water follows from faecal contamination of the supply (Feachem, et al., 1983). It has been discovered that *E. coli* is very sensitive to chlorine and other disinfectants. Therefore adequate residual disinfection should be able to eliminate any contamination in the distribution system. Waterborne outbreaks have resulted from treatment failures or from untreated water sources contaminated with faecal matter.

One of the easiest things to do to limit an *E. coli* infection is to regularly wash hands. Wash hands before handling, serving, or eating food, and especially after touching animals, working in animal environments, or using the bathroom. Practicing good hygiene and following food safety guidelines can go a long way to decreasing the risk of infection.

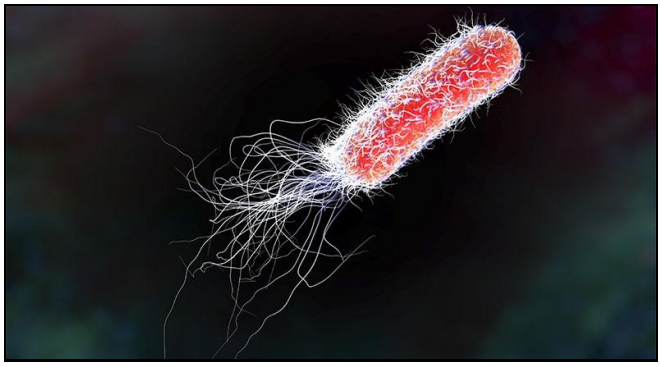

Figure 2.8: *Escherichia coli (E. coli)* (Biocote Ltd., 2016).

2.6.2 Staphylococcus aureus (S. aureus)

Staphylococci are spherical gram-positive non-spore forming bacteria, which are immobile and form grape-like clusters but can also be found singly, in pairs, tetrads or short chains (Oosthuysen, 2013). *S.aureus* is fairly widespread in the environment. The most common site of colonisation is the nose, but other sites, such as the nasopharynx, axillae and groin may also be colonised (Kloos and Bannerman, 2009). It is a normally found on skin of a healthy human being. *S. aureus* is infectious to animals and humans and can only survive on dry skin. It normally spread via contaminated surfaces, air and people (Gillaspy, et al, 2006) It is easily transmitted through air droplets or aerosol for example, when an infected person coughs or sneezes, he or she releases numerous small droplets of saliva that remain suspended in air. These contain the bacteria and can infect others. Another common method of transmission is through direct contact with objects that are contaminated by the bacteria or by bites from infected persons or animals

S. aureus is the most common cause of staph infections such as skin and soft tissue, bone and joints or organs and lungs or kidneys. However, this can easily lead to sepsis, as well as septic shock, which, in turn, is associated with vascular damage and multiple organ failure Haslinger-Loffler, et al., 2005). When *S.aureus* is detected in drinking water supplies it can easily be managed by conventional disinfection and treatment methods.

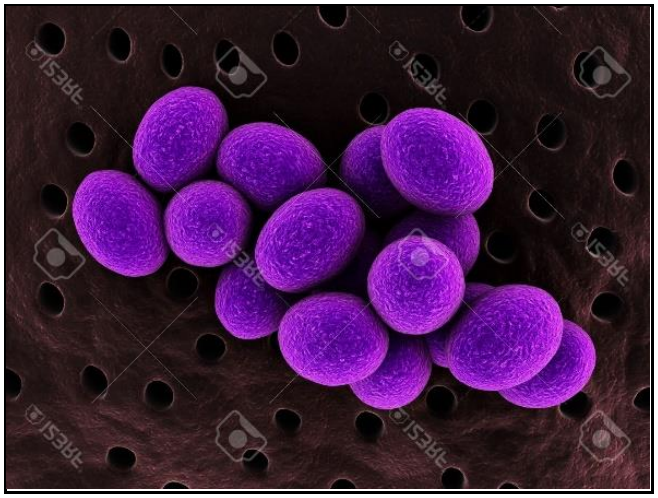

Figure 2.9: *Staphylococcus aureus* (*S. aureus*) (Drugge, 2014).

2.7 Filtration

Filters have been widely used in households and industry for the removal of substances from air and liquid. Chemical and biological contaminants present in air and water sources are an endless concern for human health. Filters for environmental protection are used to remove these pollutants from air and water for the improvement of human life (Balamurugan et al., 2011). In military, they are used in uniform garments and isolating bags to decontaminate aerosol dusts, bacteria and even viruses, while maintaining permeability to moisture vapour for comfort. Respirators are a good example for a function that requires an efficient filtration system.

Filtration systems may be improved by the use of nanofibrous media. These nonwoven filters have a pore structure which determines their properties and functions. The removal of particulate matter by a fibre based filter is determined by different mechanisms. The sieve effect comes into play by blocking large particles on the filter surface, but particles that are smaller than the surface pores will still be able to penetrate into the filter. Through interception or impaction and or static electrical attraction and these particles could still be collected by the fibres (Fang et al., 2008).

There are three important parameters which determine the diffusion, the effectiveness and the suitability of the nonwoven filters (Greiner, 2007). These are (i) overall porosity, (ii) average pore size and (iii) inner specific surface area (Mariën, 2011). Other properties that include pressure drop and flux resistance are also important factors to be evaluated for filter media.

For industries seeking to reuse their wastewater and reduce their water footprint, membrane filtration becomes the technology of choice and also the growing demand for clean water and increasing environmental concerns (Koch Membrane Systems, 2013). Membrane technology is a fast paced industry, greatly contributing to the advancement in water treatment. Because of the low operational cost, the relatively small footprint and adherence with environmental regulations are the major benefits that favour membranes over their conventional counterpart technologies, such as disinfection, distillation or media filtration (Li et al., 2017). Membrane filtration is a separation technique in which a semi permeable membrane, driven by a concentration or pressure gradient, separates two distinct phases. In comparison to conventional separation processes, a membrane is characterized by its compactness, low-cost operation and energy-efficiency (Gopal et al., 2006).

Even though electrospun nanofibrous membranes (ENMs) possess several attractive attributes that make them very attractive in separation technology, the main problem with electrospun nanofibers is that they become difficult to handle as they usually accumulate electrostatic charges during the electrospinning process (Wang et al., 2005). This would intensify as the thickness of the electrospun mesh increases. Hence, in the application of membranes, electrospun fibres require additional support to provide strength, unlike conventional cast membranes. Thus, today much of the applications of ENMs in membrane separation technology are based on hybrid systems (Shin and Chase, 2004).

Disinfection, softening, organic removal, and desalination of water and wastewater require different types of membranes that can be installed in compact, automated and modular units. These membranes are manufactured in a variety of formats such as hollow fibre, spiral, and tubular shapes. Varying degrees of separation are rendered by the different formats (Koch Membrane Systems, 2013). The hydrophilic and hydrophobic nature of a membrane material can be determined by calculating the contact angle that water makes with the membrane surface. A contact angle (θ) of greater than 90° indicates a more hydrophobic membrane material whereas a contact angle of less than 90° indicates a hydrophilic membrane material (Mecha and Pillay, 2014; Childress et al., 2012). The hydrophilicity of the membrane plays an important role in liquid filtration because it leads to adsorption of water molecules at the surface, while hydrophobicity attracts other components, increasing the membrane pollution (De Ghouy, 2010).

There are four main cross flow, pressure-driven membrane separation processes currently utilised for liquid-liquid and liquid-solid separation. Based upon the characteristic pore size and active layer chemistry or their intended application, these pressure-driven systems are commercially available as microfiltration (MF) used to filter suspended solids. Ultrafiltration (UF) mainly for virus and colloid removal. Nanofiltration (NF) for the removal of organic matter, hardness and heavy metals and lastly reverse osmosis (RO) used for desalination, water reuse and ultrapure water production (Fane et al., 2015; Werber et al., 2016). New technologies are emerging in membrane filtration which include forward osmosis (FO) and pressure retarded osmosis (PRO). Figure 2.6 shows the four separation processes.

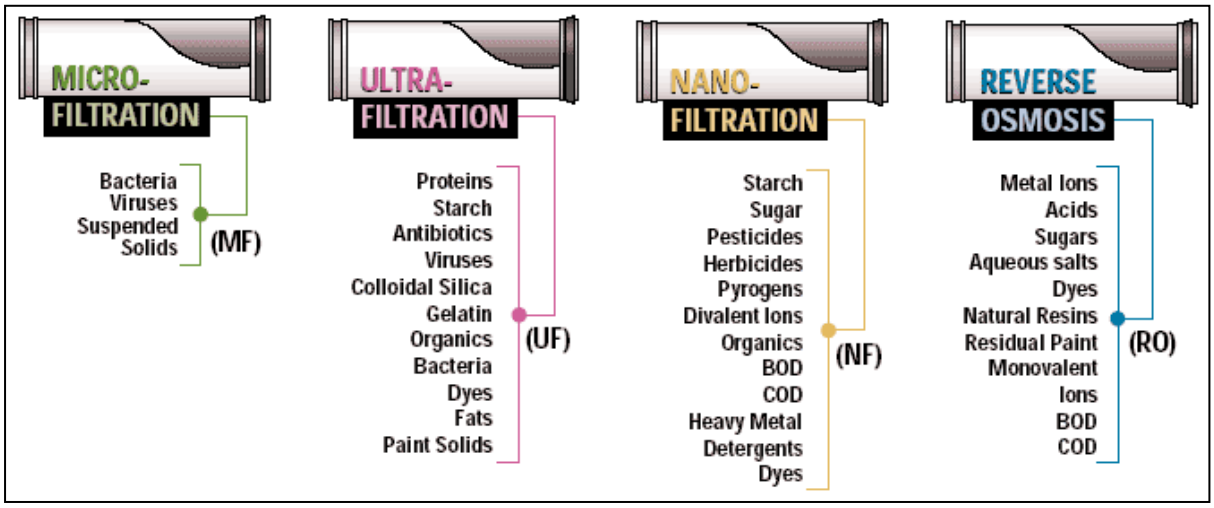

Figure 2.10: Main membrane separation processes (CHEMTRACK, Water Technology Services, 2004).

Membrane fouling is an important but unavoidable problem associated with the application of membranes. It is typically caused by inorganic and organic materials present in the feed water that adhere to the surface and pores of the membrane (Chen et al., 2016). This results in flux decline and affects the quality of the water produced. Although the performance of fouled membranes can be partially restored by appropriate cleaning methods. Chemical and physical cleaning are being used but these also have their own drawbacks. Madaeni et al., (2001) reported that most chemical cleaning ended up with the degradation of the membrane as well as corrosion in the system. Less destructive cleaning methods include hydraulic cleaning, pneumatic cleaning, and ultrasound cleaning during the process (Zhang et al., 2015) and also backwashing which is said to remove most of the reversible fouling caused by pore blocking and to partially remove loosely attached sludge cake from the membrane surface (Yigit et al., 2009). Fouling will inevitably increase operation difficulty and decrease the membrane's life

time, which will in turn lead to higher operation costs. Research is being carried out to try and mitigate this problem in membrane filtration but the development of antifouling membranes is the one getting most of the attention (Kang and Cao, 2011).

2.7.1 Microfiltration (MF)

Microfiltration systems function at low pressures and are configured based upon the application. They have larger pore sizes as compared to the other three systems mentioned above. MF is employed in dead-end filtration for water filtration, sterile bottling of fruit juices and wine, and aseptic uses in the pharmaceutical industry. MF is also used in the separation of bacteria from water known as biological wastewater treatment, separation of oil - water emulsions, used to treat water prior nanofiltration or reverse osmosis. MF membranes are specially designed not to allow micron sized substances like cells, colloids, some bacteria and suspended solids through.

2.7.2 Ultrafiltration (UF)

UF was developed not to solve problems like desalting, softening or micro pollutant removal but to replace conventional physiochemical clarification and disinfection by a physical unit (Wiesner et al., 1996). The pore size of UF membranes ranges between 5 and 20 nm. This limits the filtration to macromolecules and suspended solids, and to compounds with a molecular weight ranged from 1000 to 100000 Da (Fu and Wang, 2011). The main use of UF systems involves clarification of solutions containing suspended solids, removal of viruses and bacteria or high concentrations of macromolecules such as clarification of fruit juices, milk processing and purification of pharmaceuticals. However, in spite of its advantages, the application of membrane UF to water treatment is still limited to some degree mainly because of membrane fouling which will result in water flux decline. This results in a lot of energy consumption during production and the frequent membrane cleaning with chemicals (Nakatsuka et al., 1996).

2.7.3 Nanofiltration (NF)

NF processes fall in the transition between pure RO process that operates at high pressures and UF process that operate at low pressures. NF can be called loose RO or low pressure RO (Baker, 2004). The removal of pollutants out of effluents rests upon steric and electrical effects. The small pores and the membrane surface charge enable the rejection of charged solutes smaller than the membrane pores along with bigger neutral solutes and salts (Picq, 2015).

Nanofiltration is mainly applied in drinking water purification process steps, such as water softening, decolouring and micro pollutant removal. Other applications include the removal of pesticides from ground water, heavy metals from wastewater and waste water recycling in laundries (LENNTECH, 2014). NF presents the advantages of being easy to carry out, of being reliable and of less energy-consuming compared to RO.

2.7.4 Reverse osmosis (RO)

The reverse osmosis technique is established upon the fundamental pursuit for balance. Two liquids containing different concentrations of dissolved solids that come in contact with each other will mix until the concentration is uniform. Thin film composites (TFC) and asymmetric cellulose are the two types of RO membranes available commercially. (Li et al., 2010) reported that the TFC type shows better water flux and salt rejection, resistance to pressure compaction, wider operating temperature range and pH range, and higher stability to biological attack. Reverse osmosis is applied for the production of ultra-pure water and boiler feed water. It is also applied in the food sector (concentration of fruit juice, sugar and water). When NF and RO are used together in tandem, the function of NF is to soften the water whilst the RO's function is to polish the removal of dissolved matter and hardness (Kovacs et al., 2009).

2.8 Electrospun nanofibers in filtration

Electrospun nanofibers have been known and in use for as early as 1936 when they were still known as FP (filters of Petryanov) named after I.V.Petryanov-Sokolov whose work of fine fibre production in electrostatic fields has given the way for development of production of filter materials (Lushnikov, 1997).Advances in electrospinning methods have allowed the industrial production of more than twenty types of fibre filter materials (Shepelev and Rykunov, 1995).

In the application of water filtration membranes, nanofibers are not sufficient to withstand impacts during filtration and should be spun directly over a stronger, more rigid support e.g. melt blown supportive fibrous media (Grafe and Graham, 2003) or should be sandwiched between various layers (Wang et al., 2005). Electrospun nanofiber mats provide pronounced increases in filtration efficiency at quite small decreases in permeability. Comparing the conventional filter fibres at the same pressure drop with nanofibers with a diameter finer than half a micron, these nanofibres have a much higher capability to collect the fine particles, because the slip flow around the nanofibers increases the diffusion, interception and inertial impaction efficiencies (Kosmider and Scott, 2002). Not only solid particles can be removed by

the nanofiber mats but also liquids in an immiscible liquid system. It has been reported by (Shin et al., 2005) that electrospun polystyrene nanofibers mixed with micro glass fibres were used to remove water droplets from a water/oil emulsion. Though the capture efficiency of the filters increased, an increase in the pressure drop was observed.

2.9 Antimicrobial nanofibers (polymers)

In quite a number of areas especially in medical devices, drugs, health care and hygienic applications, water purification systems, hospital and dental surgery equipment, textiles, food packaging and food storage, microbial infection remains one of the most serious complications (Brown and Laborie, 2007). To overcome microbial infections, antimicrobial polymers can be used since it has been found out that they enhance the activity of some antimicrobial agents and minimise the environmental problems. Polymers can act as matrix of the materials holding the antimicrobial agents. Antimicrobial functional groups can be incoporated into polymer molecules in a post-polymerization modification, providing antimicrobial or antiseptic properties (Madkour et al., 2008). Characteristics of the polymer such as its hydrophilicity or its molecular weight have a great influence on the final antimicrobial activity concerning aspects from the rate of biocide release to even conferring synergistic activities (Mu˜noz-Bonilla and Fernández-García, 2012).

Self-sterilizing antimicrobial polymers are easily incorporated into fibres, extruded to fibres or electrospun into nanofibers and prevent adhesion of microorganisms to their surface. The versatility of the electrospinning process can thus generate functional nanofibrous mats that can provide more enhanced properties than those of conventional mats with lower surface-to-volume ratios (Yoon et al., 2008).

2.10 Poly (ethylene-co-vinyl alcohol)

Ethylene-vinyl alcohol (EVOH) copolymers are hydrophilic, semi crystalline materials regardless of vinyl alcohol (VOH) content consisting of ethylene and vinyl alcohol units. It has a melting point (T_m) range of 170–190 °C, depending upon the vinyl alcohol content, and a glass transition temperature (T_g) of 45–50 °C, essentially independent of vinyl alcohol content (Kenawy et al., 2003). The polymer absorbs water due to favourable hydrogen bonding interactions with –OH groups, and water acts as a plasticizer, which lowers the T_g of EVOH well below room temperature at 100 % relative humidity (Aucejo et al., 1999). They are (copolymers) prepared commercially by transesterification (saponification) of Ethylene-vinyl

acetate copolymers (EVA) (Franco-Urquiza et al., 2010). Kuraray Co., Ltd has been manufacturing and marketing EVOH copolymer resins since 1972. The company is the first and foremost producer of EVOH resins under the name EVAL™ (Kuraray Co., 2007). Via this method, EVOH with 60-75 mole % vinyl alcohol is produced. This type of EVOH has exceptional properties compared to the copolymers which are produced (via the ethylene-vinyl acetate copolymer route) as materials with high ethylene content, 82 – 90 mole %. The high ethylene content copolymers are usually used as adhesives.

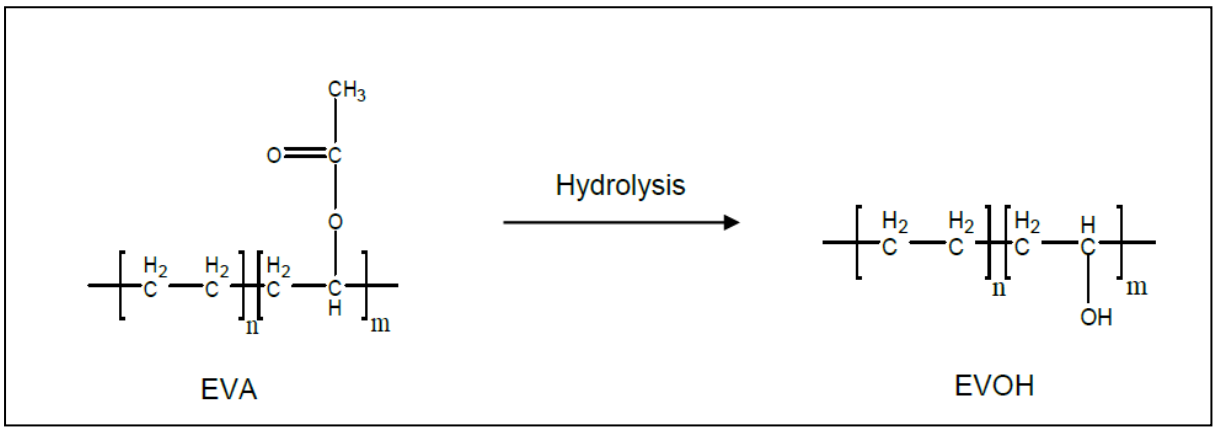

Figure 2.11: Hydrolysis of EVA to EVOH (du Toit, 2013).

EVOH is widely used because of its well-known various superior properties such as good chemical resistance, good thermal stability, good physical properties (which have great dependency on ethylene content), excellent biocompatibility, and low cost. Moreover, this polymer is generally recognized as safe (GRAS) (Lopez-Rubio et al., 2009). It has been reported that EVOH is soluble principally in DMSO and lower alcohols. It has also been found out that EVOH is soluble above 65 °C in 70/30 % v/v isopropanol/ water (i.e., typical rubbing alcohol), and that these solutions remain as such for several hours at room temperature before the EVOH begins to precipitate (Kenawy et al., 2003). The use of isopropanol / water solvent system (rubbing alcohol) as a solvent affords the opportunity to electro- spin disinfected EVOH directly onto the living tissue and this is indeed possible, suggesting an interesting approach for wound coverage and healing.

2.10.1 Properties and applications

The hydroxyl groups of the vinyl alcohol monomer units render EVOH some notable properties. EVOH copolymers are widely employed in food packaging, biomedical and pharmaceutical industries because of their excellent barrier properties to gases, hydrocarbons and organic solvents (Ramakrishnan, 1991; Alvarez et al., 2003) and excellent coextrusion

processability, while being recyclable. The excellent barrier properties can be attributed to the molecular structure of EVOH.

The presence of the hydroxyl groups in EVOH makes the polymer hydrophilic but not the ability to dissolve in water. Due to its uniform molecular structure, strong hydrogen bonds are formed within. As a result the diffusion of oxygen through the polymer becomes very difficult, since the movement of the segments that are necessary for oxygen diffusion is limited by the high inter- and intramolecular cohesive energy (Faisant et al., 1998).

Due to Resistance to chemical agents plus the barrier properties makes EVOH suitable for different industrial applications where a high-quality barrier is essential, such as chemical packaging bottles and under-floor heating pipes. In the cosmetics industry, multilayer cosmetic packages using an EVOH layer are being used since it will considerably enhance the preservation of the fragrances and ensure that the cosmetic agents maintain their effectiveness over a long period of time (Eval Europe).

Such properties are caused by strong hydrogen bond interactions, both inter and intra-molecular, which reduce the free volume of the polymer chains (Aucejo et al., 1999). The EVOH copolymer is defined by the mole % ethylene content. Lower ethylene content grades have higher barrier properties and higher ethylene content grades have lower temperatures for extrusion.

Even though it has such good properties mentioned above, EVOH does have its drawbacks. It is a water sensitive polymer and that causes problems especially in the packaging application. High humidity causes an interference with the hydrogen bonds of the polymer and weaken the interaction between the alcohol groups. Studies have shown that making EVOH nanocomposites for example incorporating clay platelets will limit access of water molecules into the polymer chains (Lopez-Rubio et al., 2005).

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Chapter 3

Experimental

3.1 Summary

The aim of this work was to prepare antimicrobial membranes for water filtration via single needle electrospinning. It has been shown that the use of biocide-containing nanofibers is effective for water sanitation (Gule, 2011), therefore emphasis was placed on the hydrophobicity of the nanofibers to see if that would affect the properties of the material in water sanitation applications. This was achieved by electrospinning three grades of poly (ethylene-co-vinyl alcohol) copolymers with different ethylene content, (i) 27 mol. % (ii) 32 mol. % and (iii) 44 mol. % ethylene content. AquaQure an aqueous antimicrobial agent containing mainly Cu^{2+} and Zn^{2+} ions was used as an additive to the polymer solutions. Investigations to determine optimum nanofibre membrane properties for water filtration application were then conducted.

3.2 Materials and methods

3.2.1 Materials

Poly (ethylene-co-vinyl alcohol) (EVOH), (27 mol. %, 32 mol. % and 44 mol. % ethylene) was purchased from Sigma Aldrich in pellet form. Isopropanol was obtained from Merck and the AquaQure biocide from AquaQure water solutions, South Africa.

3.2.2 Preparation of electrospinning solutions

Three different concentrations of polymer solutions were made up for each EVOH copolymer that is, 5 wt. %, 7.5 wt. % and 10 wt. %. This was done to determine the right concentration for optimal fibre formation to be used as filtration membranes. EVOH was dissolved in isopropanol/water (70:30 vol/vol) at 90 °C to make up the different solution concentrations. When the optimal concentration was determined, the biocide (5% vol/vol) was added to the polymer solution while stirring.

3.2.3 Electrospinning

Single needle electrospinning was employed in the fabrication of nanofibers. The horizontal setup used was designed in-house and comprises of a high voltage supply unit (0-50 kV), a syringe pump, a spinneret connected to the positive terminal as well as a grounded collector

plate connected to the negative terminal of the power supply unit. The voltage varied from 10 kV, 15 kV and 20 kV for the optimization experiments whilst the flow rate and tip to collector distance were maintained at 0.01 ml / min and 15 cm respectively.

3.3 Characterization of neat and biocidal EVOH nanofibers

3.3.1 Scanning electron microscopy (SEM) analysis

The morphology of the nanofibers was studied using the Leo® 1430VP scanning electron microscope after gold coating for 3 minutes. For each sample, images of higher magnification (X5000) and lower magnification (X1500) were taken. To confirm the presence of the biocide in the fibres, Energy dispersive x-ray spectroscopy (EDX) was done.

3.3.2 Fibre diameter measurements

The average fibre diameters of the neat EVOH and EVOH with the biocide nanofibers were measured using the Carl Zeiss AxioVisionLE 4.1 Software. Of all the measurable fibres on the image, 30 measurements were used to calculate the average diameter.

3.3.3 Attenuated total reflectance-Fourier transform infra-red spectroscopy (ATR/FTIR)

A Thermo Fisher Nicolet iS10 instrument was used for ATR-FTIR studies. A diamond crystal was fitted onto the spectrometer and measurements were taken in the 500 cm^{-1} to 4000 cm^{-1} infra-red range at a resolution of 4 cm^{-1}. Each sample spectrum was recorded after 32 scans.

3.3.4 Differential scanning Calorimetry (DSC)

Melting and crystallization behaviour of the nanofibers were investigated using differential scanning calorimetry. A Q100 thermogravimetric analyser (TA instruments) was used. According to standard procedures, calibration of the instrument was carried out with an indium metal standard. An inert atmosphere through the cell chamber was provided by a nitrogen gas purge. The method used was of three heating cycles with a fixed heating/cooling ramp of 10 °C.min^{-1}.

All six samples were heated to 200 °C to eliminate prior thermal history and held for 5 min before cooling down to 25 °C. The third step involved the reheating of the samples to the same temperature in order to obtain the crystallization and melting data.

3.3.5 Thermogravimetric analysis (TGA)

To determine the thermal stability of the nanofibers, thermogravimetric analysis was done. It was also done to see the effect of the ions on the thermal stability of the EVOH. All samples were studied using a Universal V4.1D TA Instrument. The samples were heated under nitrogen flow from room temperature to 900 °C at a rate of 20 °C.min^{-1}.

3.3.6 Water stability studies

To test the stability of the fibres in water, fibre mats of a mass of 20 mg were immersed in distilled water for 48 hours then blotted dry with tissue paper thereafter weighed to obtain the wet weight. After weighing, the fibres were further dried for a day in a vacuum oven. After drying, the nanofibers were prepared for SEM imaging to check if any morphological changes had occurred. To calculate the water absorbency value (Q_{H2O}), Equation 3.1 was used.

$$Q_{H2O} = \frac{m2-m1}{m1} \quad \text{Equation 3.1}$$

Where m_1 and m_2 are the masses before and after water immersion respectively (Liu et al., 2009).

3.3.7 Contact angle measurements

The hydrophobicity of nanofibrous mats was measured using static contact angle measurements. For this experimental part, nanofibers had to be melt pressed into films. A 1 µL drop of distilled water was placed onto the sample film. The static contact angles were measured using Carl Zeiss AxioVisionLE 4.1 Software. The contact angles obtained are an average of 12 measurements. Figure 3.1 shows the captured image of a water droplet with the marked parameters that were needed to calculate the contact angle. Equation 3.2 was used to determine the static contact angle.

$$\theta = 2\times tan^{-1}\left(\frac{H}{R}\right) \quad \text{Equation 3.2}$$

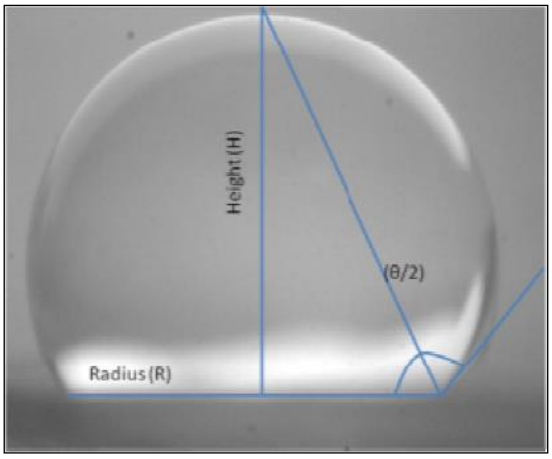


Figure 3.1: Static contact angle parameters needed for determining the contact angle (Reader, 2011).

3.4 Results and discussion

3.4.1 Optimization of Electrospinning conditions

In order to find the right average diameter of the nanofibers to be used in the study, two parameters which have an effect on the resultant fibres were investigated. These parameters are the concentration of the polymer solutions and the voltage whilst the tip-collector distance (15 cm) and the flow rate (0.010 ml/min) remained unchanged. The results are summarized in Tables 3.1, 3.2 and 3.3.

3.4.2 Effect of solution concentration and voltage applied

According to literature, the properties of the polymer solution greatly influence both the electrospinning process and the resultant fibre morphology (Angammana, 2011). One such property is the solution concentration which affects the viscosity of the solution. If the viscosity of the solution is high, then the polymer chain entanglements are also high, and vice versa. In the electrospinning process, for fibre to be formed a minimum amount of polymer chain entanglement, i.e., viscosity, is required. If the solution concentration is high, the fibres obtained will have a high fibre diameter and beaded fibres can also be observed.

For the polymer jet to initiate from the Taylor cone, either a positive or a negative voltage of more than 6 kV is required (Taylor, 1964). An increase in the applied voltage is known to lead to a decrease in the diameter of the electrospun nanofibers. This is due to the increase in the resultant electric field between the needle and the target leading to greater stretching of the

solution because of the larger Columbic force between the surface charges (Mo et al., 2004; Lee et al., 2004; Supaphol et al., 2005).

From the results presented in Table 3.1 to Table 3.3 below, it can be seen that with an increase in solution concentration the average diameters of the fibres obtained was high and this correlated with literature. Zhao et al., 2004 reported that a lower voltage leads to a weaker electric field, which reduces the speed of the jet and in turn increases the flight time of the electrospinning jet, thus resulting in thinner fibres. However, there was no linear relationship observed with an increase in voltage. With an increase in voltage the nanofiber diameters had varying average diameters.

Table 3.1: 27 mol. % - varying solution concentration and voltage

Wt.%	**Average diameters(nm) at (x) voltage**		
	10 kV	**15 kV**	**20 kV**
5	238.8	201.8	337.8
7.5	310.5	314.1	354.4
10	481.9	478.5	505.6

Table 3.2: 32 mol. % - varying solution concentration and voltage

Wt.%	**Average diameters(nm) at (x) voltage**		
	10 kV	**15 kV**	**20 kV**
5	323.2	276.2	348.3
7.5	421.3	392.5	393.8
10	476.6	430.3	416.9

Table 3.3: 44 mol. % - varying solution concentration and voltage

Wt.%	**Average diameters(nm) at (x) voltage**		
	10 kV	**15 kV**	**20 kV**
5	328.9	348.7	292.2
7.5	370.1	374.7	370.4
10	396.8	395.1	387.8

A large aspect ratio is one of the characteristics which give nanofibers their outstanding properties. Smaller fibre diameters results in a large aspect ratio and this gives better filtration efficiency. Bearing this in mind, the optimal spinning conditions used in this study were a concentration of 5wt. %, voltage of 10 kV, working distance of 15 cm and a flow rate of 0.010 ml/min.

Fibre diameters of the nanofibers with the biocide were measured to see if the ions present would influence the resultant fibre diameters. The results in Table 3.4 show that smaller average fibre diameters were obtained, due to the biocide making the polymer solutions more conductive. To form the nanofibers, the repulsion of the charges at the surface of the electrospinning jet causes the solution to elongate. When the electrical conductivity of the solution is increased a notable reduction in the diameter of the electrospun nanofibers can therefore be observed because the jet carries more charges.

Table 3.4: Comparison of average diameters of neat EVOH and EVOH/AqQ nanofibers

Polymer	**Neat EVOH**	**EVOH/AqQ**
27 mol. %	310.5 nm	213.1 nm
32 mol. %	323.2 nm	265.7 nm
44 mol. %	328.9 nm	272.8 nm

The difference in morphologies of the neat EVOH nanofibers and that of the EVOH/AqQ can be seen in Figure 3.2.

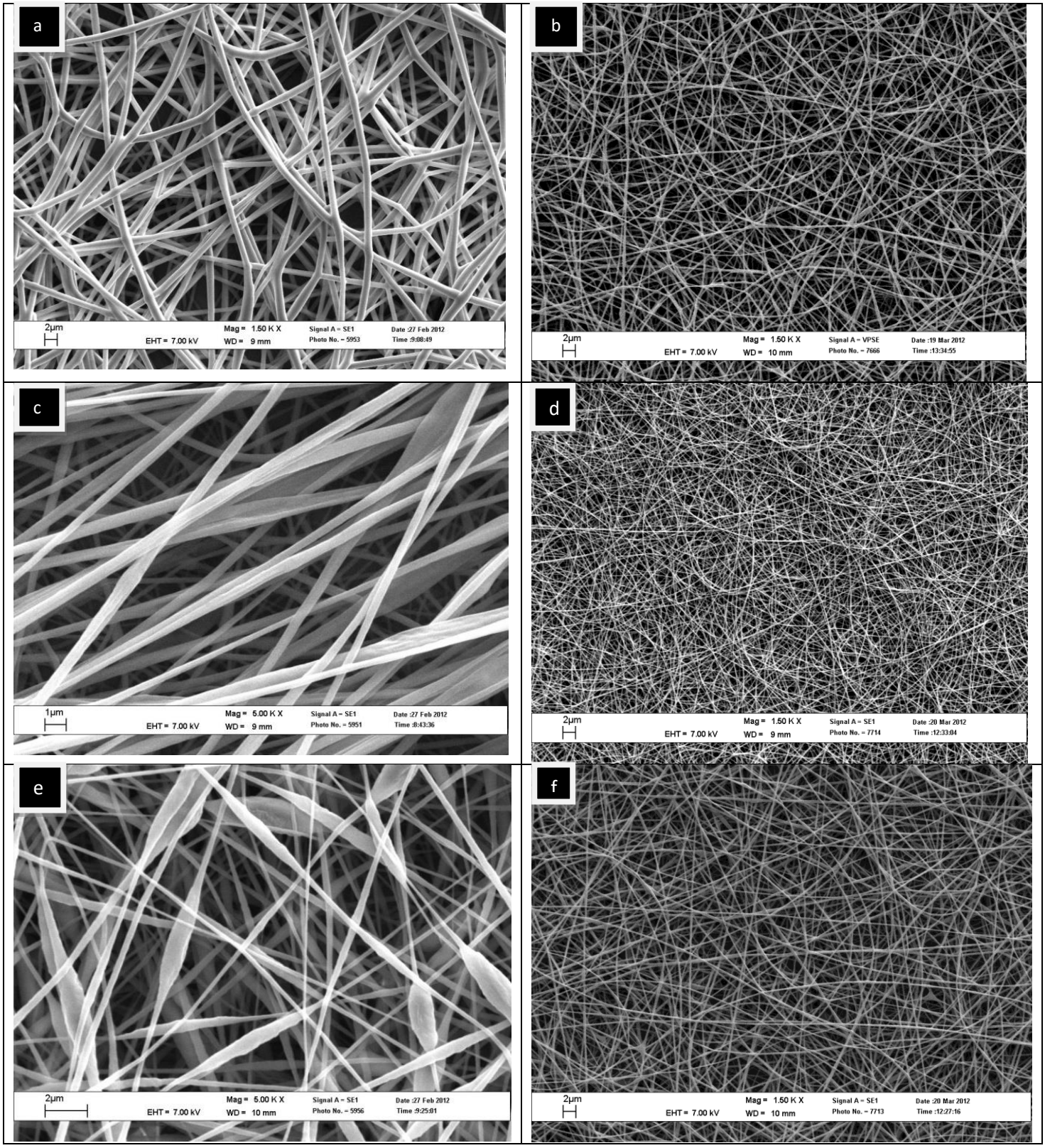


Figure 3.2: SEM Images showing the difference between neat EVOH (a- 27 mol. %, c-32 mol. % and e- 44 mol. %) and EVOH/AqQ (b- 27 mol. %, d- 32 mol. % and f- 44 mol. %) nanofibers.

3.4.3 Energy dispersive X-ray analysis (EDX)

Energy dispersive x-ray spectroscopy was done to confirm the incorporation of the biocide into the polymer solutions hence resulting in modified nanofibers. Figure 3.3 shows the spectrum obtained from the analysis of the 27 mol. % EVOH with the biocide included. Copper, zinc, iron and potassium peaks can be seen on the spectrum because the biocide is an aqueous solution of several metal ions. Copper is the most abundant in the biocide and the results correlate with that. Same results were obtained for the 32 mol. % and 44 mol. % EVOH with the biocide.

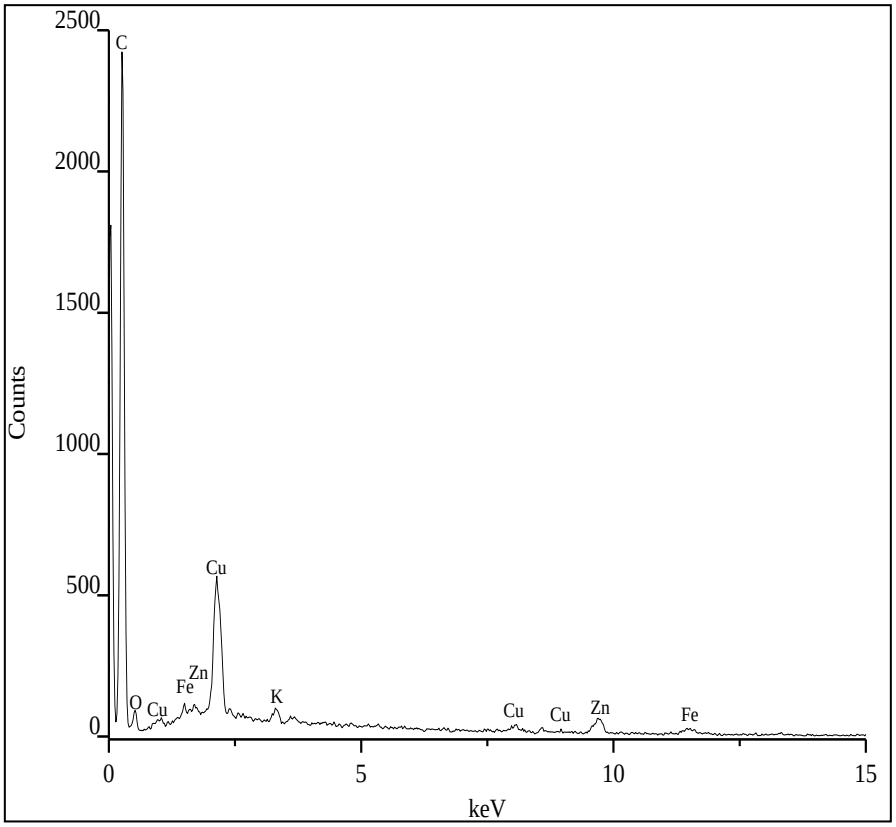


Figure 3.3: EDX Spectrum of EVOH/AqQ - 27 mol. % nanofibers

3.4.4 Attenuated total reflectance-Fourier transform infra-red spectroscopy (ATR-FTIR)

ATR-FTIR graphs of the neat fibers and the modified fibers did not show much difference except for the shift of the OH band from 3320 cm^{-1} in neat EVOH fibers to 3330 cm^{-1} in EVOH/AquaQure fibers. This shift in the OH peak suggests its interaction with the metal ions. This is due to the chelating capabilities of the hydroxyl groups to retain the metal ions (Lin et al., 2002). Otherwise the prominent EVOH peaks can be seen on the spectra; OH (3320cm^{-1})

as mentioned earlier, CO (1460 cm^{-1}) and the -CH_2-CH_2- stretches in the (2850-2960 cm^{-1}) region and (837 cm^{-1}). The spectrum below is similar to the other spectra which are not shown.

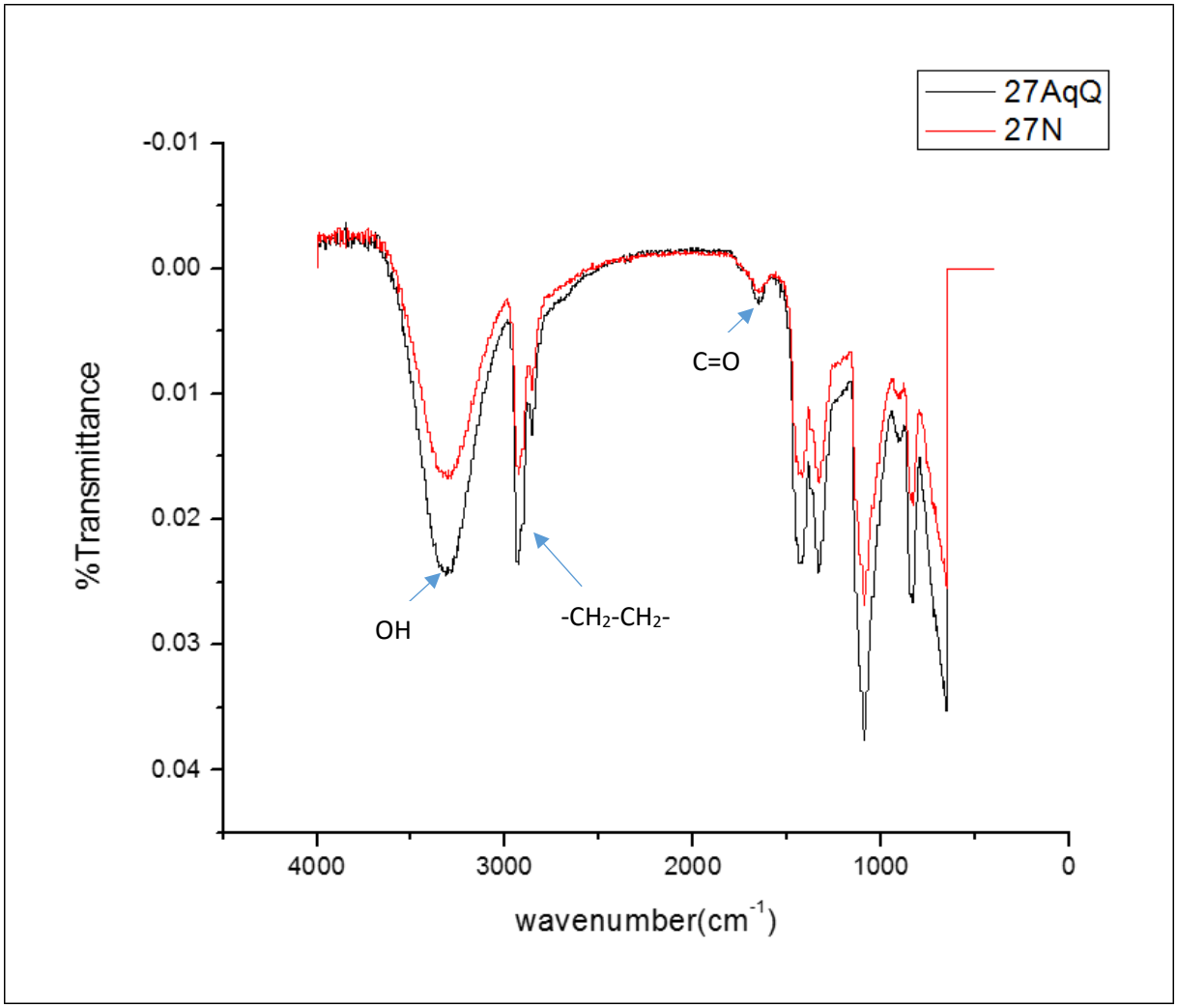


Figure 3.4: FTIR overlay of neat 27 mol. % and 27 mol. %/AqQ nanofibers

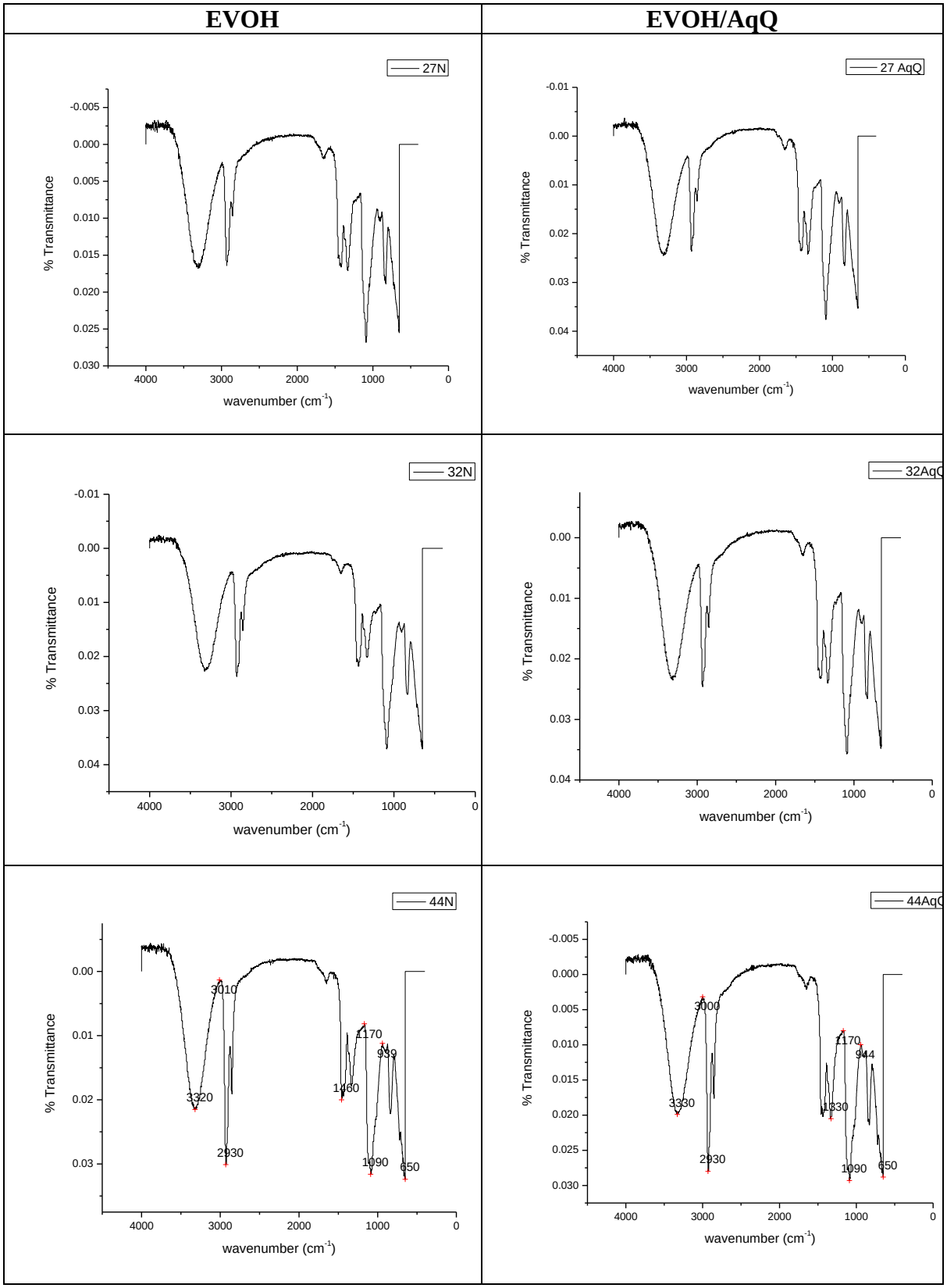


Figure 3.4.1 FTIR spectra of the neat EVOH (27, 32 and 44 mol. %) and EVOH/AqQ (27, 32 and 44 mol. %) nanofibers

3.4.5 Thermogravimetric analysis (TGA)

Thermogravimetric analysis was done to determine the thermal stability of the fabricated nanofibers and to establish the effect of AquaQure on the stability of the fibres. Thermal degradation process of the neat and modified nanofibers can be well illustrated with DTG curves shown in Figures 3.5, 3.6 and 3.7.

For both the neat 27 mol. % EVOH and 27 mol. % EVOH with AquaQure, three degradation steps can be observed in Figure 3.5. The first step (1) with a peak maximum at 200 °C is due to loss of water and other volatile substances such as residual solvent. Decomposition of the alcohol groups occurs in the second step (2) with a peak maximum at 350 °C. Chemical degradation of the fibres occur in the last step (3) with a maximum at 450 °C (Diedericks, 2016).

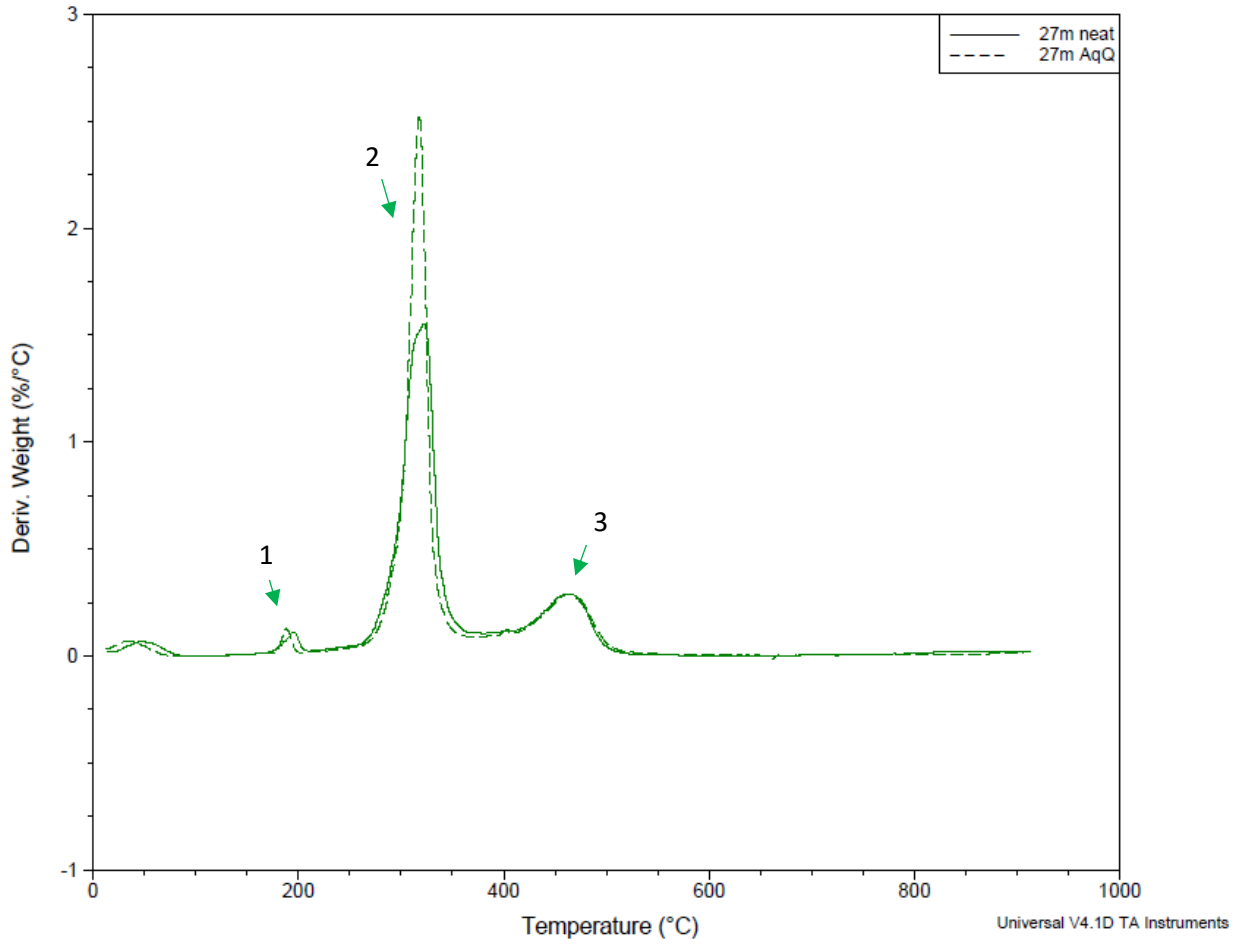


Figure 3.5: Derivative TGA curve for 27 mol. % EVOH

The DTG curve of the modified 27 mol. % EVOH shows a sharp peak in the second step where the alcohol groups decompose. This is due to the chemical interaction of the hydroxyl groups with the metal ions. The sharpness of the peak suggests strong interactions since there are many hydroxyl groups in the 27 mol. % EVOH.

Three degradation stages were also observed for the 32 mol. % EVOH. The DTG curve of the modified 32 mol. % shows a peak shift from 400 °C to 310 °C in the second stage (2) of degradation. The addition of the biocide disrupts the crystalline structure and hence reducing the thermal stability of the polymer.

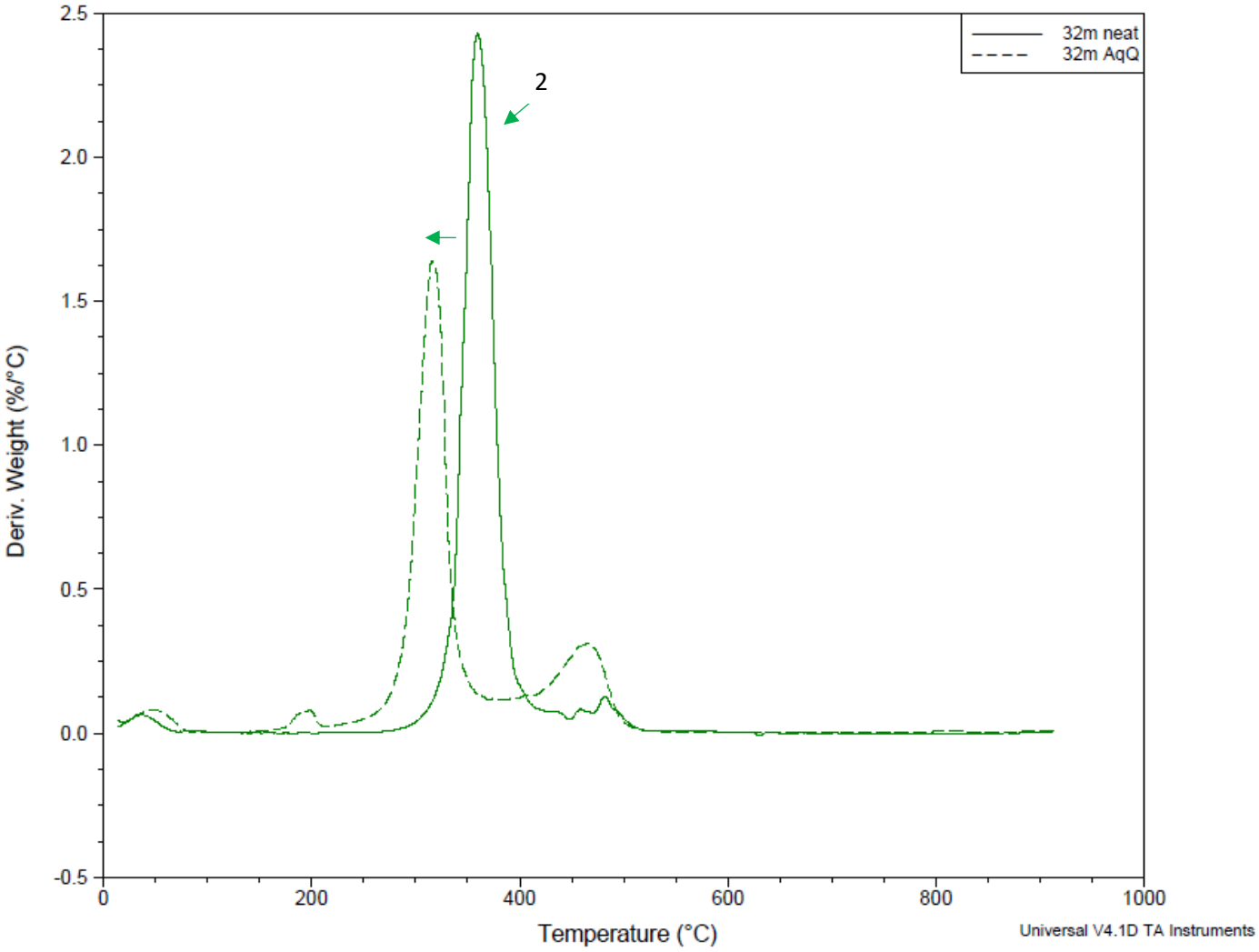


Figure 3.6: Derivative TGA curve for 32 mol. % EVOH

Similar results were obtained for the 44 mol. % EVOH. It seems that there was more disruption that occurred in the 44 mol. % compared to the 32 mol. % and 27 mol. % as can be seen in Figure 3.7 below. The degradation peak maximum at 400 °C shifts to 280 °C.

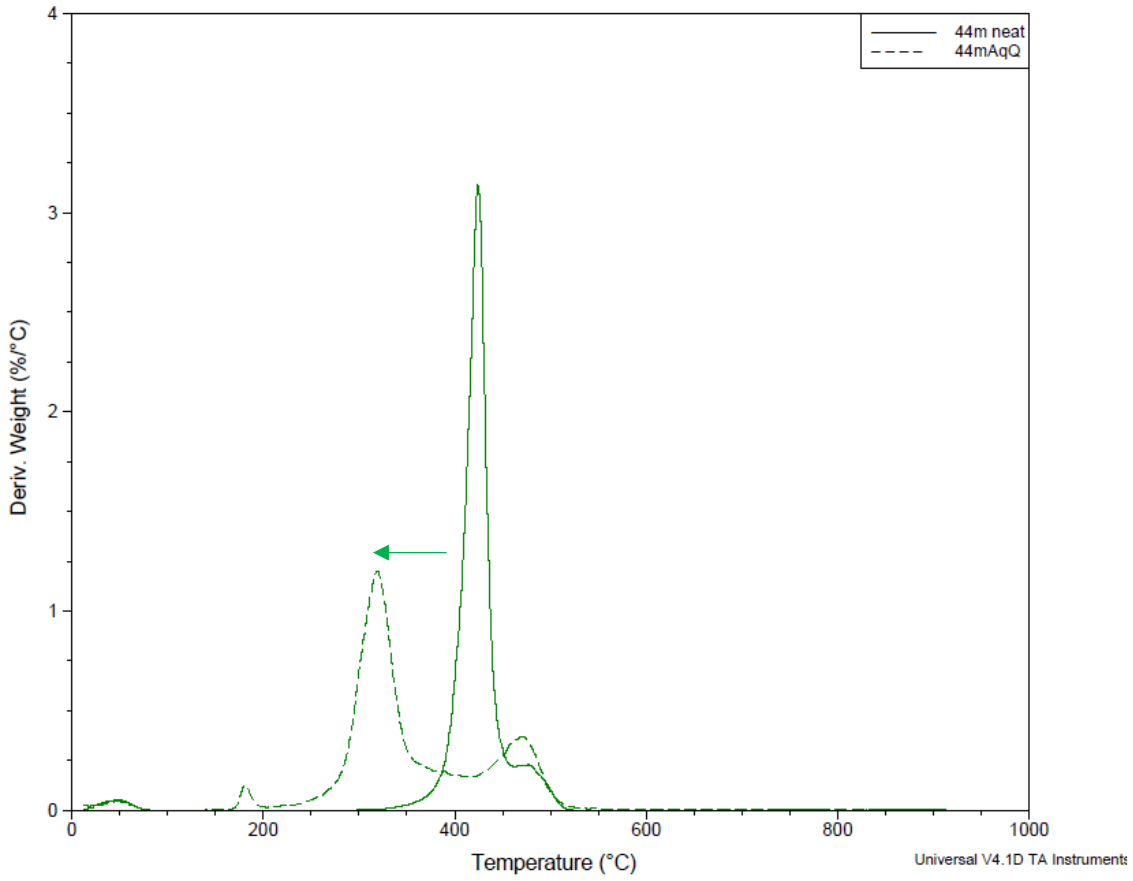


Figure 3.7: Derivative TGA curve for 44 mol. % EVOH

Therefore, the addition of the metal ions decreased the thermal stability of the 32 mol. % and 44 mol. % EVOH whilst the 27 mol. % remained unchanged.

3.4.6 Differential scanning calorimetry (DSC)

Table 3.5 displays the DSC results of the neat and modified nanofibers. It shows the thermal properties of the nanofibers before and after the incorporation of metal ions. It was observed that all the temperatures decreased with either an increase in ethylene content or with the addition of the metal ions.

Table 3.5: DSC results

Polymer	***Tg* (°C)**	***Tc* (°C)**	***Tm* (°C)**	***X_c* (%)**
27N	91.84	165.71	187.79	43.72
27AqQ	70.43	130.94	165.34	32.40
32N	87.82	161.15	182.37	28.82
32AqQ	69.62	113.30	150.39	31.46
44N	64.31	144.17	163.70	20.22
44AqQ	56.67	88.51	133.56	26.97

As the ethylene content increases, less hydrogen bonding exists between the polymer chains. From 27 mol. % to 44 mol. %, the glass transition, crystallisation and melting temperatures decrease. With the addition of the biocide, the crystalline structure of the polymers is reduced. This is supported by the tabulated percentage crystallinity (X_c (%)) results hence a decrease in the melting and crystallisation temperatures is observed. Though Shi et al., (2011) reported that the interactions between the metal ions and the polymer hinder chain movements leading to an increase in the Tg, this was not the case in our study. TGA and DSC show that the incorporation of the metal ions affects the thermal properties of the EVOH polymers.

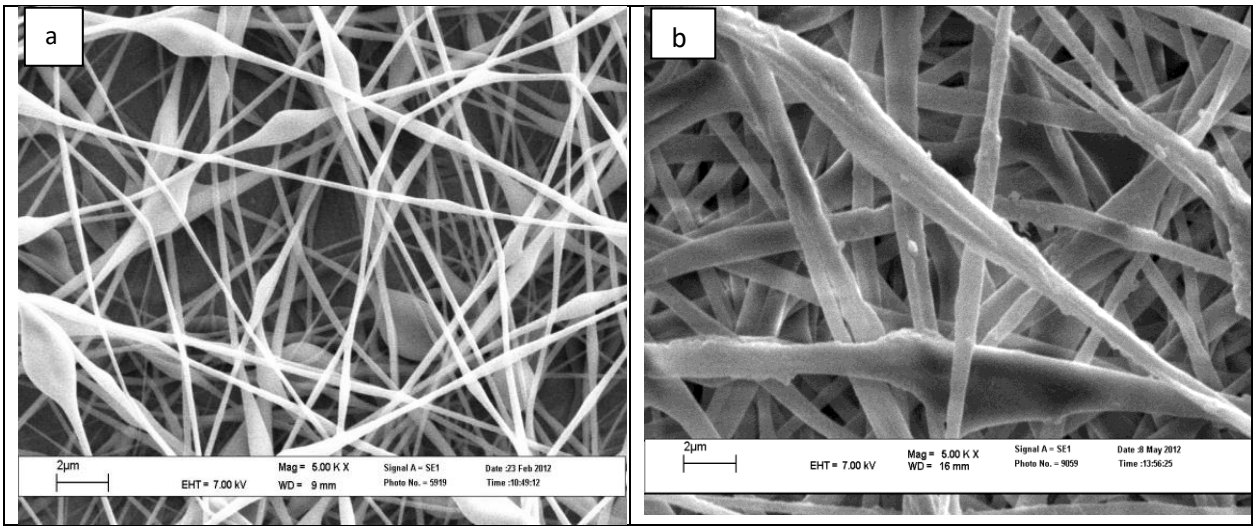
3.4.7 Water stability studies

Using Equation 3.2, the water absorbency (Q_{H2O}) was calculated and the results are summarised in Table 3.6. From the calculated Q_{H2O} values, 27 mol. % absorbed more water compared to the 32 mol. % and the 44 mol. %. This is an expected trend because the more available –OH groups the more they bond with the water molecules. The modified nanofibers absorbed less water compared to the neat nanofibers. This is so because there are less available –OH groups to bond with the water molecules since the hydroxyls are chelating with the metal ions.

Table 3.6: Water absorbency results

Polymer	Water absorbency (Q_{H2O}) (g/g)
27N	2.21
27AqQ	1.84
32N	1.62
32AqQ	1.41
44N	1.38
44AqQ	1.13

From SEM images it can be seen that the fibres become flatter after they have been put in water. They still maintain their fibre structure which shows that they are stable in water. Further investigations need to be done to find a way to control the flattening of the fibres as this could compromise the membrane's lifecycle in water filtration applications.



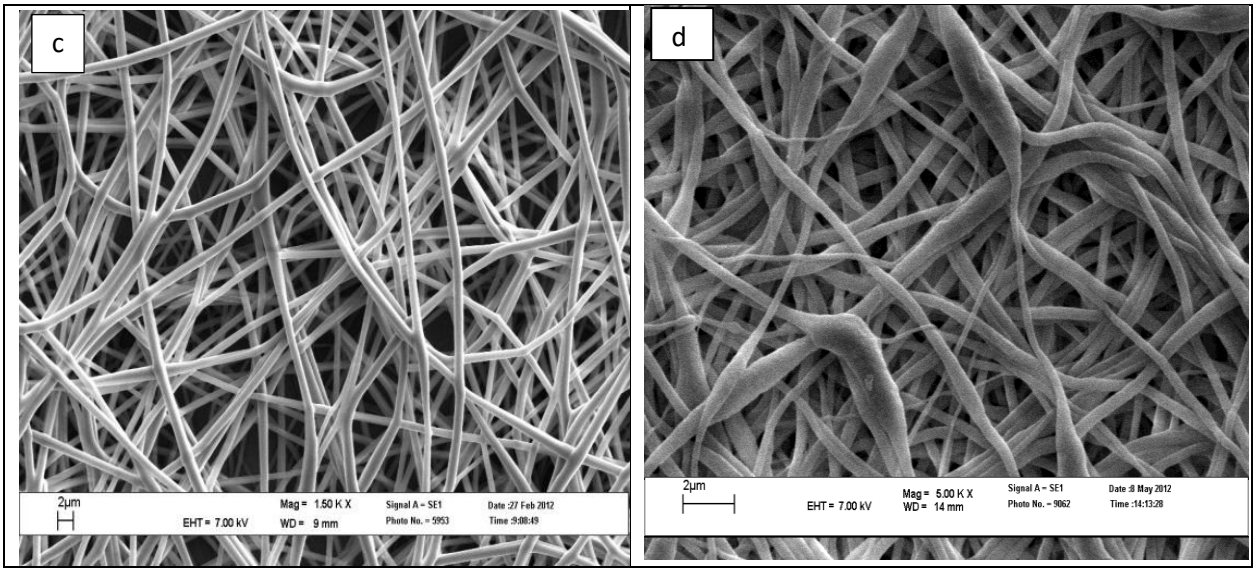


Figure 3.8: Water stability studies. (a) neat EVOH before immersion in water, (b) neat EVOH after immersion in water, (c) EVOH/AqQ before immersion in water and (d) EVOH/AqQ after immersion in water.

3.4.8 Contact angle measurements

The contact angle measurement is an indication of how wet a solid material gets when in contact with a liquid. Small contact angles of < 90° suggest high wettability, while large contact angles of > 90° suggest low wettability (Yuan et al., 2013). Many polymers exhibit hydrophobic surfaces. Highly hydrophobic surfaces made of low surface energy may have water contact angles as high as ~120° (Zisman, 1964). Some materials with highly rough surfaces may have a water contact angle even greater than 150°, due to the presence of air pockets under the liquid drop. These are called super hydrophobic surfaces.

Table 3.5: Static contact angles

Polymer	***θ(°)***
27N	73.31 ± 2.64
27AqQ	85.41 ± 8.87
32N	81.16 ± 5.93
32AqQ	116.55 ± 9.16
44N	84.76 ± 3.84
44AqQ	97.91 ± 9.11

With an increase in ethylene content the contact angle becomes larger indicating a hydrophobic surface. This is an expected result, more ethylene groups mean less of the hydroxyls which form the hydrophilic part of the polymer. The modified nanofibers show an increased contact angle which suggests that the presence of metal ions within the polymer matrix increases their hydrophobicity. The ions have a chelating effect in the presence of hydroxyl groups, and because of this interaction the resulting nanofibers become more hydrophobic compared to their neat versions. 32AqQ has the largest contact angle which suggests that there is more interaction between the 32 mol. % and the ions in the AquaQure but this is expected of the 27 AqQ instead of the 32 AqQ therefore further investigations have to be done in order to get an explanation as to why the modified 32 mol. % has an extremely larger contact angle of 116° and not the 27AqQ whose θ is 85°. According to literature contact angles greater than 90° indicate that the surface is highly hydrophobic and it can be concluded that the 32AqQ and 44AqQ are highly hydrophobic.

3.5 Conclusion

The nanofibrous mats, neat EVOH and modified EVOH/AquaQure were successfully fabricated using the conventional single needle electrospinning. It was observed that the higher the solution concentration the greater the average fibre diameter. However, unexpected results were obtained when the voltage was varied. The higher the voltage, the greater the average fibre diameter but this observation is supported in literature. The incorporation of the biocide was confirmed by EDX and ATR-FTIR. The modified nanofibers diameters were also measured and it was observed that with the ions present, the average fibre diameters were lower than those of the neat nanofibers. The ions made the solutions more conductive hence smaller diameters were obtained.

TGA showed that the decomposition of the nanofibrous membranes occurs in three steps. The metal ions decreased the thermal stability of the neat EVOH fibres. Tg, Tc and Tm temperatures decreased with an increase in ethylene content as well as with the incorporation of the metal ions.

Water stability studies were carried out to see if these fibres maintained their integrity after being exposed to water for long periods of time. The calculated Q_{H2O} decreased with an increase in ethylene content. The addition of the metal ions also decreased the water absorbency of the fibres. It was observed that both neat and modified fibre mats remained coherent though

they became a bit flatter. To check the influence of the metal ions on the hydrophobicity of the fibre mats, static contact angles were measured. From the results obtained, the more hydrophobic polymer, 44 mol. % had the greatest contact angle. The ions seem to increase the hydrophobicity of the nanofibers mats but the 32 EVOH/AquaQure had an outstanding value making it almost a super hydrophobic material. The fabricated nanofibers do have a potential to be used for water filtration applications.

3.6 References

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Chapter 4

Experimental

4.1 Part A: Antibacterial and release studies of Poly (ethylene-co-vinyl alcohol) (EVOH) nanofibers are discussed in this chapter

4.1.1 Summary

In this chapter the filtration of contaminated water using the antimicrobial nanofibrous membranes is explained as well as the release rates of the metal ions from the modified EVOH nanofibers. The antimicrobial activity of the EVOH/AqQ fibres against bioluminescent strains of *Staphylococcus aureus* - Xen 36 and *Escherichia coli* - Xen 14 was tested using plate counting and confirmed with bioluminescent imaging. Bioluminescent imaging quantifies the cells that enter a dormant state during contact with the antimicrobial fibres and this is done to eliminate the chances of overestimating the antimicrobial efficiency of the nanofibers.

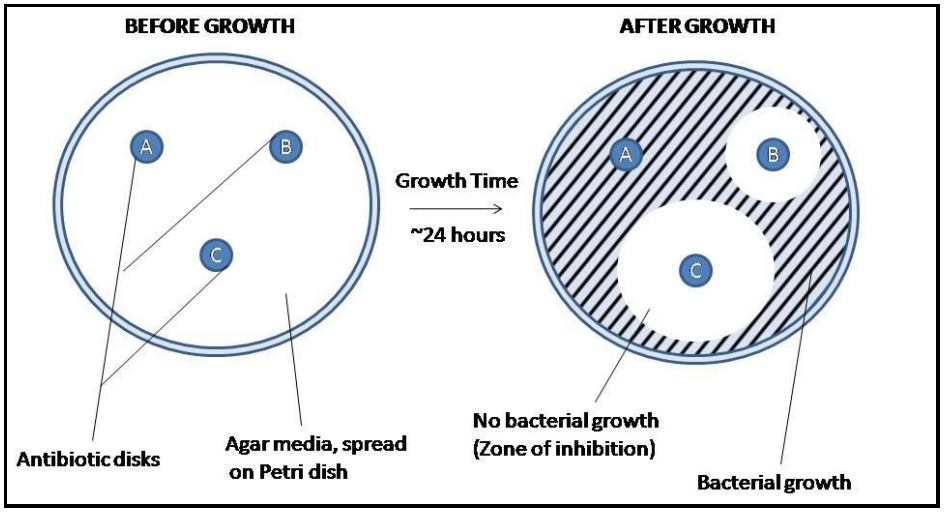


Figure 4.1. Zones of inhibition around a test sample (Sommer, 2016).

Also as part of determining the effectiveness of the antimicrobial filters, zone of inhibition tests were done adapting the Kirby-Bauer disk-diffusion method. The pathogens of interest are swabbed uniformly across a culture plate. Then a filter-paper disk, saturated with the compound to be tested, is placed on the surface of the agar. The compound diffuses out from the filter paper into the agar (Johnson and Case, 1995). If considerable antimicrobial activity is present then a zone of inhibition will be observed around the test sample. The zone of inhibition can be defined as the area on the agar plate that remains free from microbial growth. Figure 4.1, above, shows the concept.

Inductive Coupled Plasma –Atomic Emission Spectroscopy (ICP-AES), Ultra Violet visible (UV/Vis) Spectroscopy and Conductivity measurements were done to establish the release profile of the metal ions from the fabricated membranes which would explain the performance of a particular membrane in the water sanitation application intended. This would serve as a way to determine if there was any significant leaching out of metal ions from the membranes during the filtration process.

4.2 Materials and method

4.2.1 Antimicrobial tests

Two strains of bacteria were used, Gram-positive *Staphylococcus aureus* - Xen 36 and Gram-negative *Escherichia coli* - Xen 14 (Caliper Life Sciences, Hopkinton, MA, USA). These two strains are modified with the luxABCDE operon on the bacterial chromosome in order for them to bioluminesce when metabolically active. 10 mL Brain Heart Infusion (BHI) broth (Biolab Diagnostics) with the appropriate antibiotics was used as a culture for the bacteria. The culture was left overnight on a rotating wheel at 37 °C. Cells were pelleted by 10 min centrifugation at 3000 rpm, and washed 3 times with physiological water for each strain. Spiked water samples were prepared by inoculating 10^6 CFU/mL of each pathogen into 500 mL sterile physiological water.

The neat EVOH and EVOH/AquaQure nanofibrous membranes were placed on 0.22 µm pore filters (Whatman® filters). The spiked water was filtered through the fabricated nanofibrous membranes as illustrated in Figure 4.2. The filter pore size of 0.22 µm excluded bacterial cells, causing the cells to remain in contact with the fibres.

The nanofibers were then rinsed three times using 10 mL saline water to wash off all remaining bacterial cells and the wash-off water was plated out on BHI agar plates to determine living cells. This was done after specific contact periods (0 minutes which is immediate contact, 30 and 60 minutes) to determine cell death as a function of time. After incubating the plates overnight at 37 °C, the number of bacterial colony forming units (CFU) was counted. The counts were used to calculate the surviving number of bacteria thus giving an understanding of the antibacterial efficacy of the filters. The degree of antibacterial efficacy is the ratio of reduction of bacterial colonies. The equation is given by;

$$\%R = [(A-B)/A] \times 100 \qquad \text{Equation 4.1}$$

Here, %R = Reduction rate, A = CFU before filtration and B = CFU after filtration with the functionalised nanofibers (Lala et al., 2007).

Soon after filtration, each sample was fixed in 2.5% (v/v) glutaraldehyde in PBS at 4°C for 4 hours. The fibres were allowed to air dry and were prepared for SEM imaging by fixing on carbon adhesive tape and sputter coating with gold.

Bioluminescent imaging (BLI) was done using XENOGEN VIVO VISION In Vivo Imaging Lumina System (IVIS) supplied by Caliper Life Science in order to check cell viability by quantifying total photons emitted by the cells.

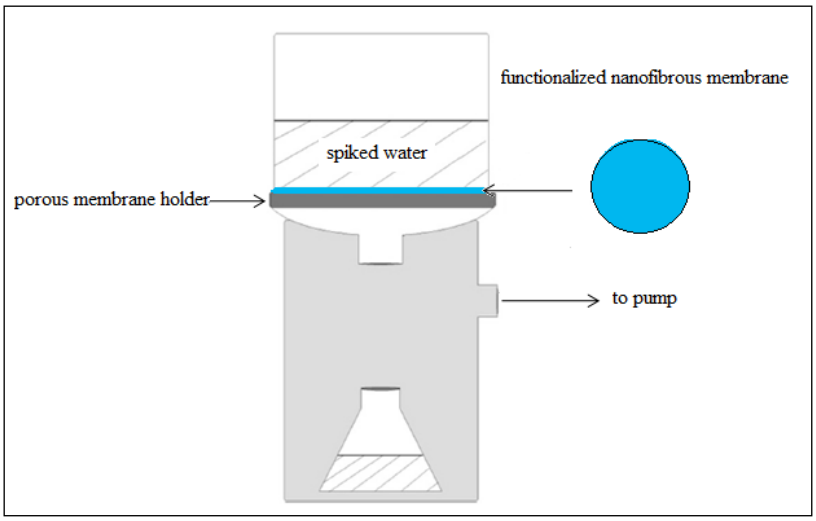


Figure 4. 2: Experimental filtration setup (Daels et al., 2011).

4.2.2 Zone of inhibition tests

Two plates were prepared for *Staphylococcus aureus* and *Escherichia coli*, plate (i) as a control and plate (ii) antibacterial material on the agar. The plates were incubated overnight at 37 °C. The diameter of the clear area in millimetres was measured by a ruler. Three separate measurements for each material 27AqQ, 32AqQ and 44AqQ were obtained. In addition BLI was done to check cell viability by quantifying total photons emitted by the cells. The plates were kept covered at all times. The data obtained was interpreted using the guidelines in Table 4.1 below.

Table 4.1: Literature values of Inhibition zone diameters and conclusions (Johnson and Case, 1995).

	Inhibition zone diameter (mm)
Unsusceptible	≤ 10
Moderate	11–15
Susceptible	≥ 16

4.2.3 Inductive Coupled Plasma –Atomic Emission Spectroscopy (ICP-AES) and Ultra Violet visible (UV/Vis) Spectroscopy

Preliminary tests showed that most of the ions are released from the membranes after three hours therefore 100 mg of nanofibers were immersed in 100 ml distilled water. 20 ml aliquots were taken at 30 minute intervals for up to three hours. The Thermo ICap 6200 ICP-AES was used to measure major and minor elements down to mid-ppb values and the Agilent 7900 ICP-MS was used to measure the ultra-trace elements. Absorbance measurements were carried out on a Perkin Elmer Lambda 20 UV-Vis Spectrometer.

4.2.4 Conductivity tests

Leaching of AquaQure from the nanofiber membranes was determined by measuring conductivity. 50 mg of each material was placed in 10 ml of demineralised water and the conductivity was measured against time.

4.3 Results and Discussion

4.3.1 Plate counting after filtration

The antibacterial activity of the pristine EVOH and EVOH/AquaQure nanofibers was compared against *S. aureus* and *E.coli*.

S. aureus

Antibacterial activity was observed with immediate contact of the *S. aureus* with the neat 27 mol. % EVOH membrane. This was observed for the neat 32 mol. % and 44 mol. % EVOH membranes. This indicated that EVOH has some interesting biological activities which need to be further investigated. This observation could be attributed to the residual solvent in the nanofibrous membranes. The solvent system used was isopropanol/water (70:30 vol/vol) and this is commercially known as rubbing alcohol (Widmer, 2000). Caldwell, 2017 explained that rubbing alcohol kills bacteria by disrupting the cell membrane. The lipids that are part of the

outer protective cell membrane of each bacterium cell are made more soluble in water by the alcohol so that the cell membrane begins to lose its structural integrity and fall apart. A linear relationship was observed between the contact time and log reduction. Up to 2.69 log reduction (Figure 4.3) after 60 minutes for the 27 mol. % EVOH membrane was observed and for the 32 mol. % (Figure 4.5) and 44 mol. % (Figure 4.5), 3.59 and 3.71 log reduction respectively was observed after 60 minutes. The neat 44 mol. % EVOH membrane had the highest log reduction of viable cells compared to the neat 27 mol. % and 32 mol. %.

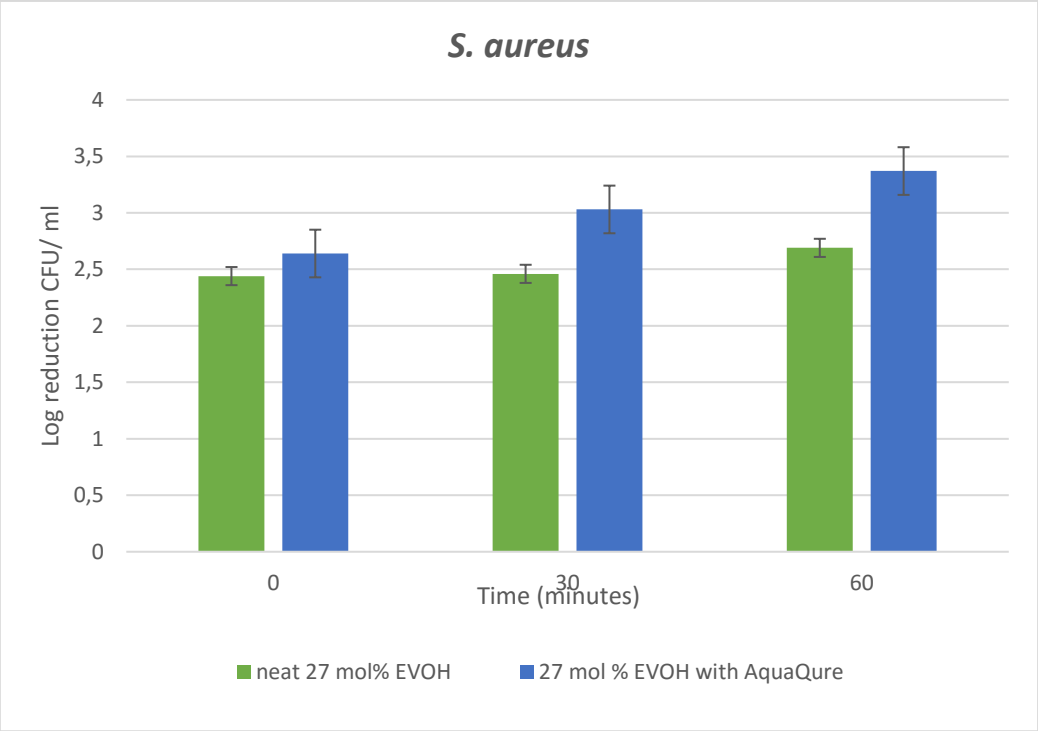


Figure 4.3: *S. aureus* CFU counts for 27 mol. % membrane

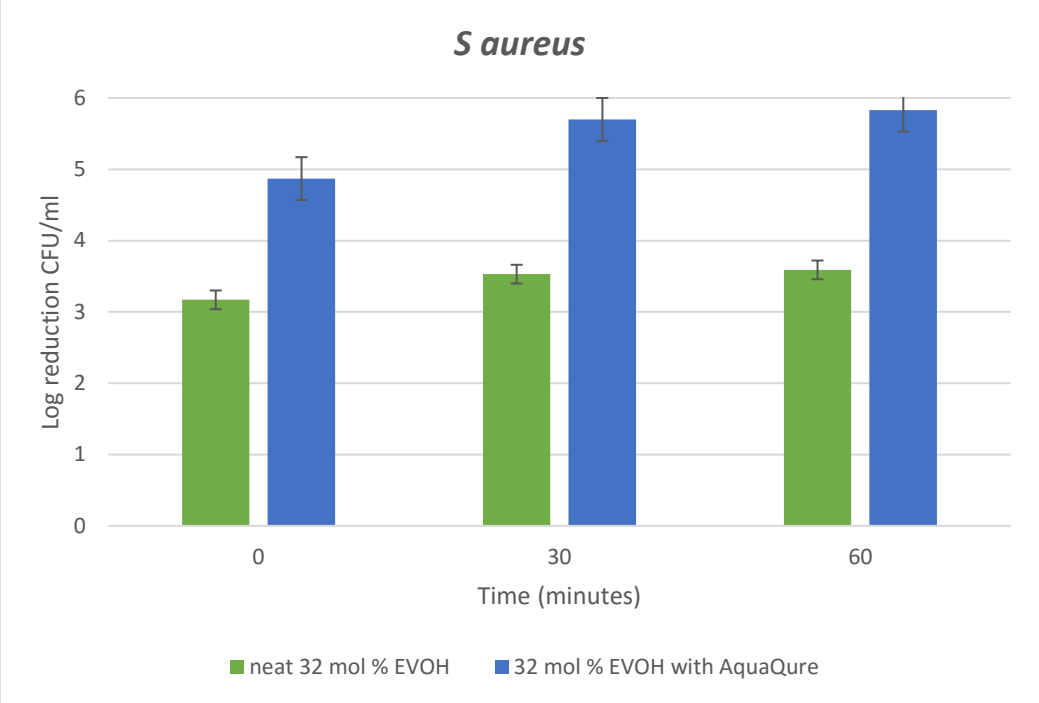


Figure 4.4: *S. aureus* CFU counts for 32 mol. % membrane

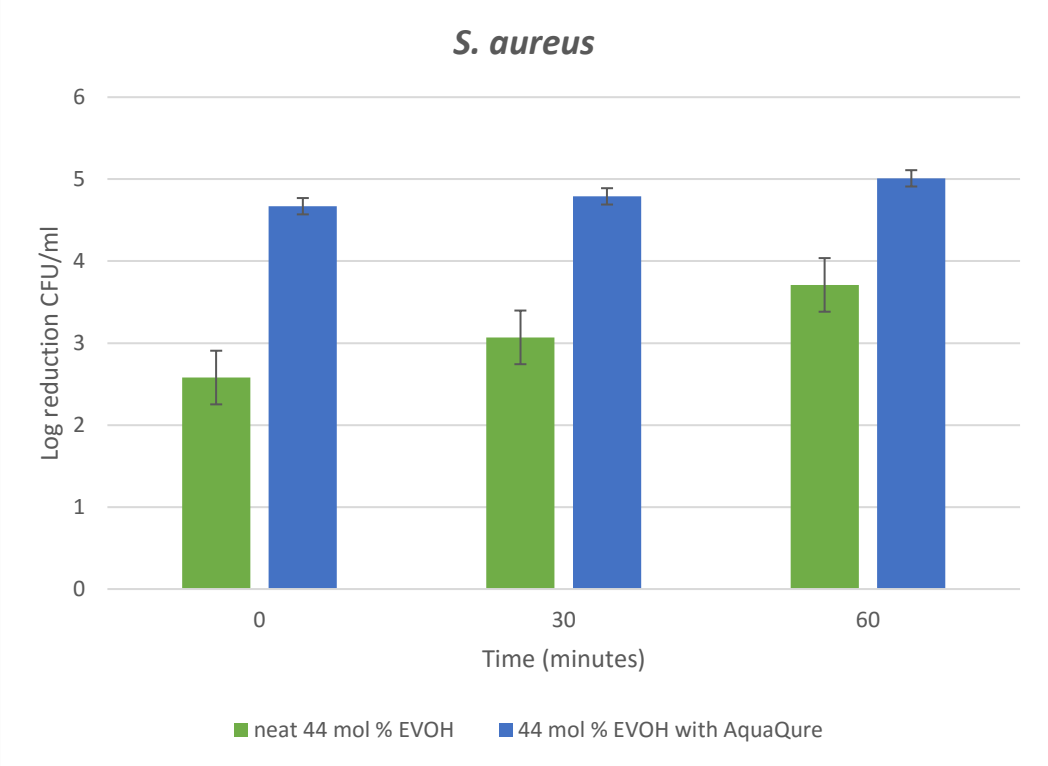


Figure 4.5: *S. aureus* CFU counts for 44 mol. % membrane

Increased antibacterial activity was observed from the EVOH membranes with AquaQure. Log reductions of 3.37 (27 mol. %), 5.83 (32 mol. %) and 5.01 (44 mol. %) were obtained after 60 minutes. With increase in contact time, more bacteria were deactivated. It was curious to observe that the 32 mol. % EVOH treated with AquaQure was more effective in deactivating the *S. aureus* bacterial strain and not the 27 mol. % EVOH. In order to inactivate or kill microbes, the metal ions must come into contact with the microbes. An and Friedman (1998) reported that bacteria with hydrophilic properties such as *S. aureus* would prefer a hydrophilic surface to adhere to. The 27 mol. % EVOH is the most hydrophilic of the three and yet it exhibited the least antibacterial efficacy.

E. coli

Figures 4.6, 4.7 and 4.8 show the results obtained when *E. coli* spiked water was filtered through the nanofibrous membranes. Neat EVOH membranes showed antimicrobial activity against *E. coli*. The log reduction of viable cells increased with an increase in contact time. The 27 mol. % had 1.85, 32 mol. % and 44 mol. % had 2.35 log reduction after 60 minutes.

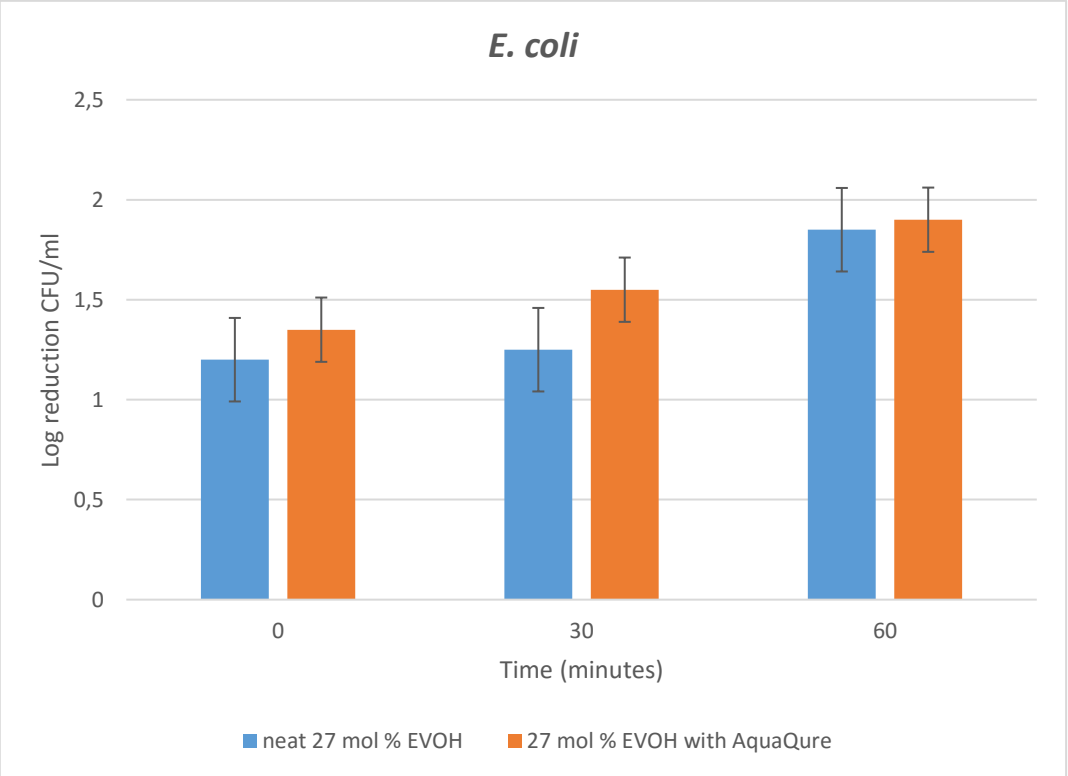


Figure 4.6: *E. coli* CFU counts for 27 mol. % membrane

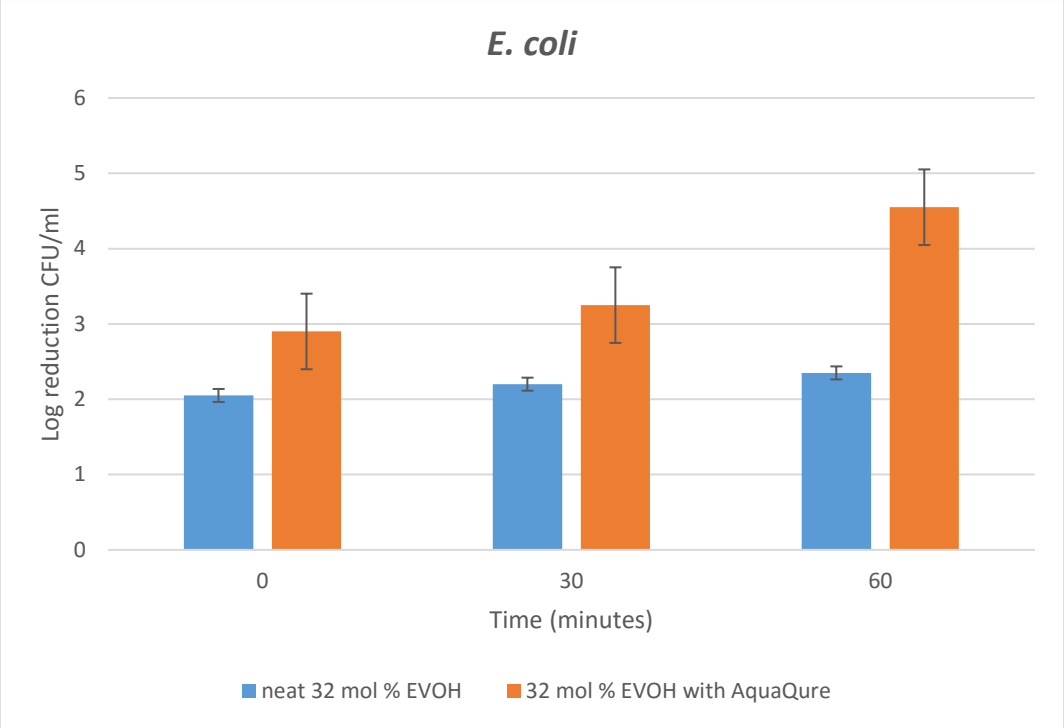


Figure 4.7: *E. coli* CFU counts for 32 mol. % membrane

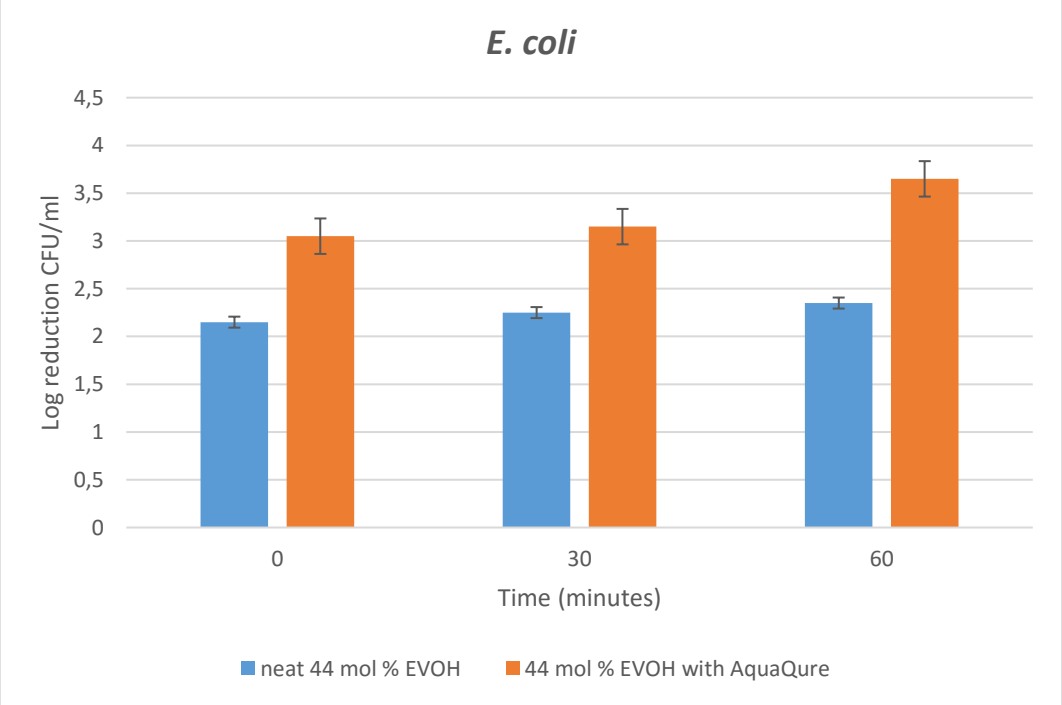


Figure 4.8: *E. coli* CFU counts for 44 mol. % membrane

The membranes with AquaQure showed an increase in antimicrobial activity. Log reductions of up to 4.55 were achieved by the 32 mol. % EVOH membrane after 60 minutes and it exhibited the most antibacterial activity once again against the *E. coli* bacterial strain.

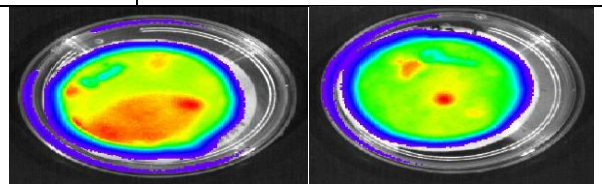
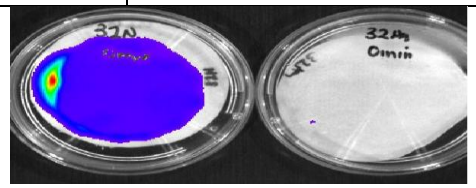
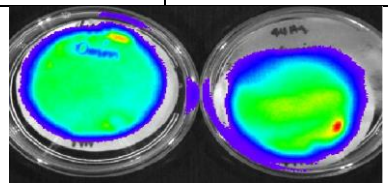
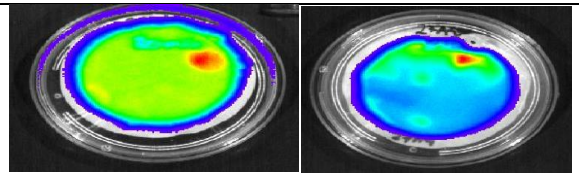
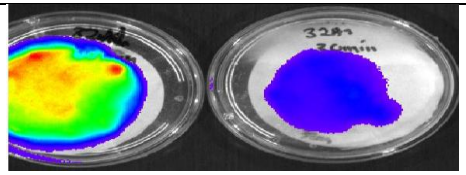
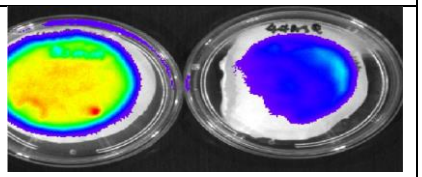
From these plate count experiments, the fabricated membranes deactivated the *S. aureus* more than the *E. coli*. Cell wall structural differences between these classes of bacteria i.e. Gram positive and Gram negative bacteria results in susceptibility differences (Singariya et al., 2012).

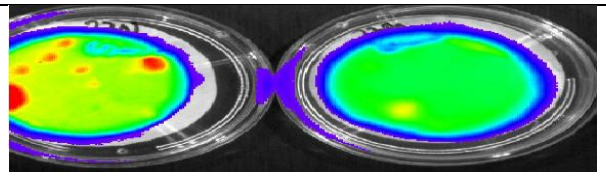
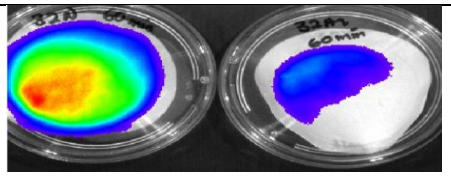
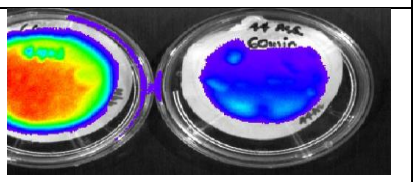
4.3.2 Bioluminescence imaging (BLI)

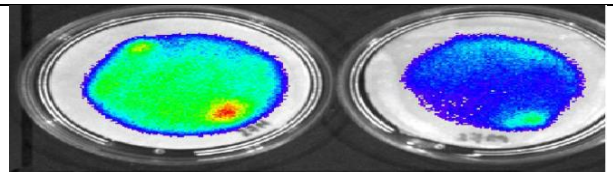
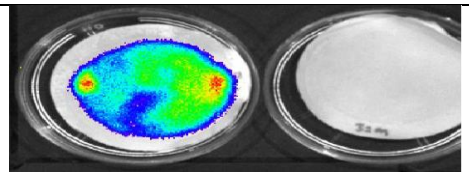
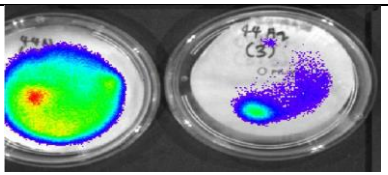
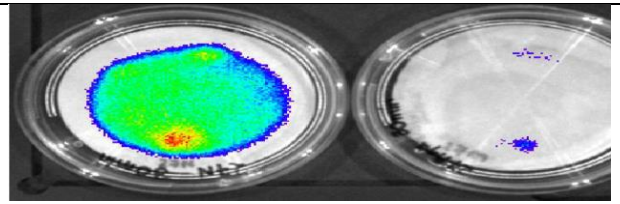
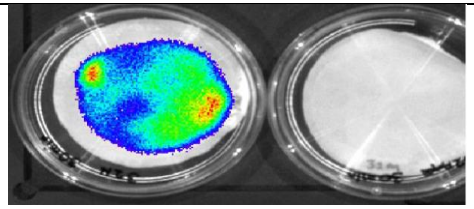
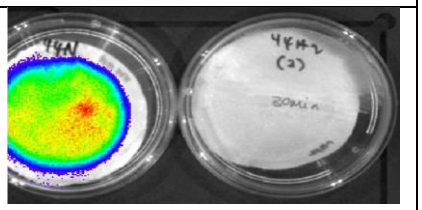
From the bioluminescence images in table 4.2 it can be seen that the fabricated membranes are indeed antibacterial because of the visible loss of bioluminescence after the bacterial cells have been in contact with them. After filtration through the neat EVOH membranes there is a reduction of viable cells which is an indication of the antibacterial activity of the neat EVOH fibres. These findings correlate with the plate count results. When filtered through the EVOH AquaQure nanofibrous membranes there was a percentage reduction ranging between 56- 99% CFU depending on contact time of the cells with the membranes for the two strains tested. This suggested that the loss of bioluminescence was related to the decrease in viable cells. As can be seen from the images below, the membranes were more effective in deactivating the Xen-36 strain as compared to the Xen-14 strain since there is little bioluminescence on some membranes and none on others.

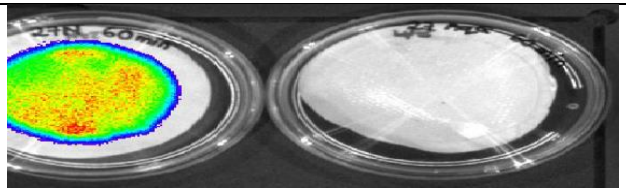
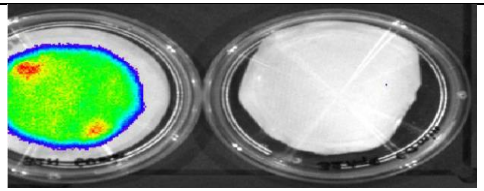
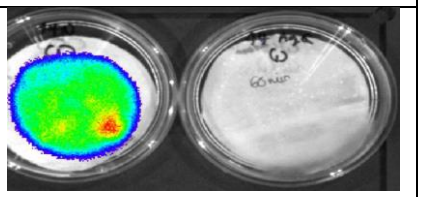
Looking at the individual membranes, it can be seen that with more contact time between the bacterial cells and the membranes, more cells are deactivated. Comparing the three types of nanofibers independent of the contact time, the 32 mol. % EVOH exhibits the best properties for efficient water sanitation.

Table 4.2: Bioluminescence Images showing antibacterial activity of the nanofibrous membranes

Strain		**27 mol. %**		**32 mol. %**		**44 mol. %**	
		EVOH	**EVOH/AqQ**	**EVOH**	**EVOH/AqQ**	**EVOH**	**EVOH/AqQ**
Escherichia coli (Xen 14) **0 min**							
CFU (average of triplicates)	Before filtration (A)	2.45×10^{9}		2.45×10^{9}		2.45×10^{9}	
	Filtration with EVOH	1.46×10^{9}		1.90×10^{8}		1.60×10^{8}	
	Filtration with EVOH/AqQ (B)	1.07×10^{9}		1.36×10^{7}		2.60×10^{7}	
%Reduction in CFU %R=[(A-B)/A]×100		56.33%		99.44%		98.93%	
30 min							

CFU (average of triplicates)	Before filtration (A)	2.45×10^{9}	2.45×10^{9}	2.45×10^{9}
	Filtration with EVOH	1.24×10^{9}	1.20×10^{8}	1.03×10^{8}
	Filtration with EVOH/Aq Q (B)	8.60×10^{8}	1.30×10^{7}	1.97×10^{7}
%Reduction in CFU %R=[(A-B)/A]×100		64.90%	99.47%	99.20%
60 min				
CFU (average of triplicates)	Before filtration (A)	2.45×109	2.45×109	2.45×109
	Filtration with EVOH	3.05×108	2.05×107	7.00×107
	Filtration with EVOH/Aq Q (B)	2.34×108	8.80×105	4.00×106
%Reduction in CFU %R=[(A-B)/A]×100		90.45%	99.96%	99.84%

Staphylococcus aureus (Xen 36) **0 min**				
CFU (average of triplicates)	Before filtration (A)	2.24×10^{10}	2.24×10^{10}	2.24×10^{10}
	Filtration with EVOH	8.20×10^{7}	1.50×10^{7}	5.90×10^{7}
	Filtration with EVOH/Aq Q (B)	5.10×10^{7}	3.00×10^{5}	4.80×10^{5}
%Reduction in CFU %R=[(A-B)/A]×100		99.77%	99.99%	99.99%
30 min				
CFU (average of	Before filtration (A)	2.24×10^{10}	2.24×10^{10}	2.24×10^{10}
	Filtration with EVOH	7.80×10^{7}	6.60×10^{6}	1.90×10^{7}

triplicates)	Filtration with EVOH/Aq Q (B)	2.10×10^{7}	4.50×10^{4}	3.60×10^{5}
%Reduction in CFU %R=[(A-B)/A]×100		99.91%	99.99%	99.99%
60 min				
CFU (average of triplicates)	Before filtration (A)	2.24×10^{10}	2.24×10^{10}	2.24×10^{10}
	Filtration with EVOH	4.60×10^{7}	5.70×10^{6}	4.40×10^{6}
	Filtration with EVOH/Aq Q (B)	9.60×10^{6}	3.30×10^{4}	2.20×10^{5}
%Reduction in CFU %R=[(A-B)/A]×100		99.96%	99.99%	99.99%

4.3.3 SEM imaging after filtration

Morphological changes of the bacterial cells after filtration were viewed using the scanning electron microscope. Xen-36 (Gram-positive) showed structural changes when in contact with the biocidal membranes whilst the Gram-negative did not. The observed disintegration in the cell wall membrane could be due to the imbalance in the osmotic pressure (Sheikh et al., 2011). Kim et al., 2015 suggested that the Gram-negative bacterial cell wall outer membrane acts as a barrier to many substances, including synthetic and natural antibiotics.

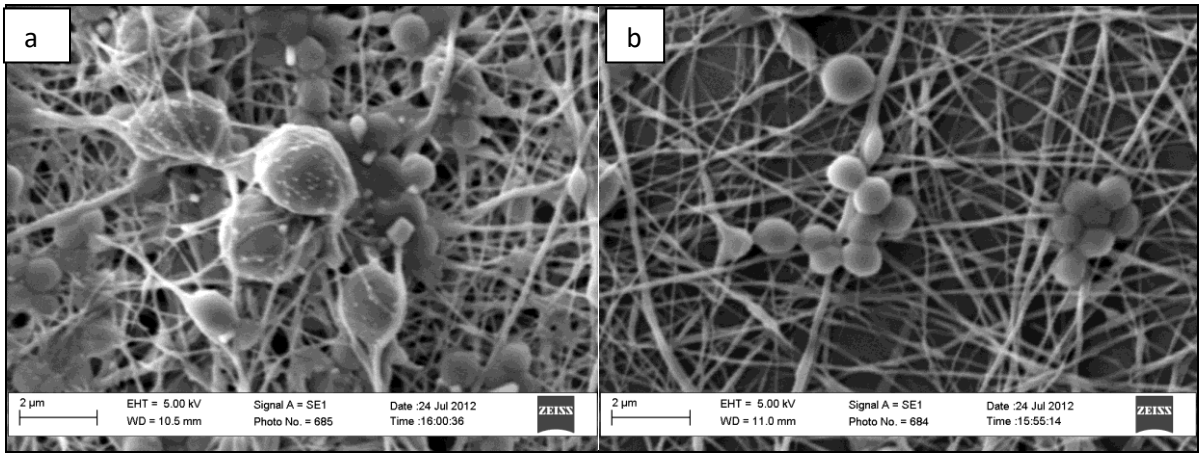


Figure4.9: SEM Images of *S. aureus* after filtration. (a) Intact bacterial cells on 32mol% EVOH membrane and (b) lysed bacterial cells on 32mol%/AqQ membrane.

4.3.4 Zone of inhibition tests

Zone of inhibition testing is known to be a fast and inexpensive way used to determine antimicrobial activity relative to other laboratory tests. If there is considerable antimicrobial activity, a zone of inhibition is observed around the test sample.

BLI was done to see if there were zones of inhibition around the membranes. The loss of bioluminescence around the membranes suggested the inhibition of growth on the plates after incubation. The zones of inhibition measurements were taken using the BL images. The values recorded are an average of three measurements.

Table 4.3: Zone of Inhibition (mm)

Sample	Zone of Inhibition (mm)	
	E.coli	***S.aureus***
27AqQ	15	12.8
32AqQ	16.9	14
44AqQ	17.4	13.5

The EVOH with Aquaqure membranes managed to inhibit growth of bacteria around them as can be seen from the results in table 4.3. Referring to table 4.1 it can be concluded that these two bacterial strains are susceptible to the AquaQure biocide. For the *S. aureus*, the 32 AqQ membrane had the largest average zone of inhibition and this correlates with the plate counting results. The average zone of inhibition for the *E. coli* increased with the increase in hydrophobicity of the membrane suggesting that the hydrophobicity influenced the way in which the biocide is released from the membrane. The 27 AqQ remained the least effective membrane. Antimicrobial agents that leach out of the object and into the aqueous agar matrix, such as silver ions, usually show better results than antimicrobials that stay affixed to the object therefore release studies of the biocide from the membrane had to be done.

4.3.5 Inductive Coupled Plasma-Atomic Emission Spectroscopy, ICP-AES (Release profiles)

ICP-AES is routinely used to analyse major, minor and some trace elements in water, experimental solutions, rocks and so forth. An inductive coupled plasma is used to produce excited atoms from a sample. These emit electromagnetic radiation at wavelengths characteristic of a particular element. Then a spectrometer separates and resolves these lines and measures their ionic strength.

For the fabricated membranes to be suitable for use in the water sanitation application, the release of the biocide should be rapid. As the contaminated water gets into contact with the membrane, the bacteria has to be deactivated immediately. Inductive coupled plasma- atomic emission spectroscopy was done as a way of investigating the rate at which the biocide is released by the membranes during filtration. A decision was made to show copper and zinc release profiles since they are the most abundant metal ions in the biocide used in this study.

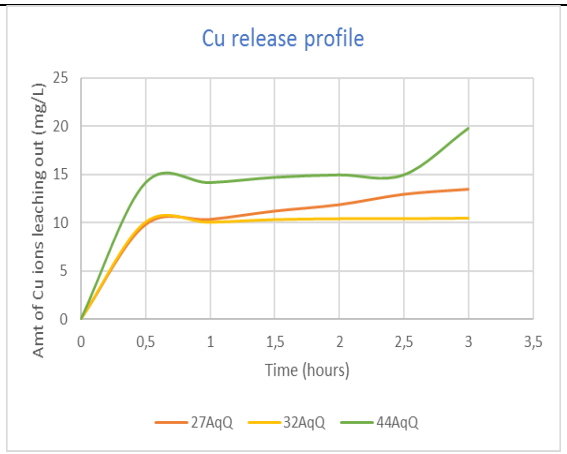


Figure 4.10: Copper release profiles of the 3 membranes

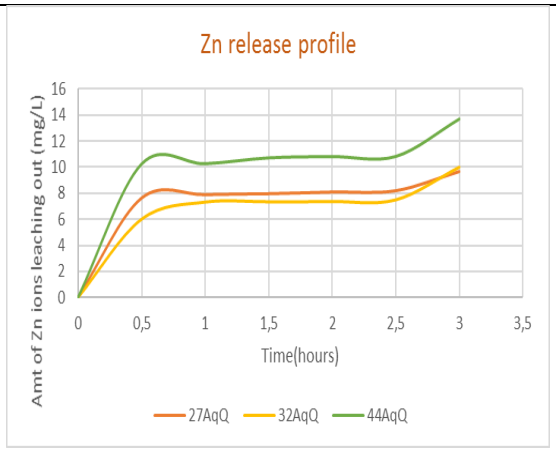


Figure 4.11: Zinc release profiles of the 3 membranes

The release of the metal ions is dependent upon the interaction of the metal ions with the polymer and the interaction between the bacteria and the nanofibrous membrane. The copper and zinc release rate results are shown in Figures 4.10 and 4.11 respectively.

Comparing the three membranes, the results show that the 44AqQ membrane releases the ions at a faster rate than the 32AqQ and the 27AqQ. The 44AqQ membrane which is the most hydrophobic of the three, has less chemical interactions with the metal ions hence their fast release from the membrane. There are not enough hydroxyl groups to chelate with the ions. This correlates with the zone inhibition results. The 44AqQ membrane has the largest zone of inhibition especially against the E.coli.

From CFU counts it was found out that the 32AqQ had the most antibacterial efficacy with almost a hundred percentage reduction regardless of the contact time of the bacteria and the membrane. But according to the obtained release profiles, the 32AqQ membrane releases the metal ions at the slowest rate. Even though its hydrophobicity is intermediate, it seems to have quite a large degree of interaction with the ions which is comparable to that of the27AqQ membrane.

4.3.6 Conductivity measurements

Conductivity is a property of ionic solutions. It is defined as the ability of a substance to transmit heat, electricity or sound. The charges on ions in solution facilitate the flow of electrical charge therefore the conductivity of a solution is proportional to its ion concentration. The conductivity value gives a rapid and inexpensive way to determine the ionic strength of a

solution. Electrical conductivity is a measure of the saltiness of the water and is measured on a scale from 0 to 50 000 µS/cm.

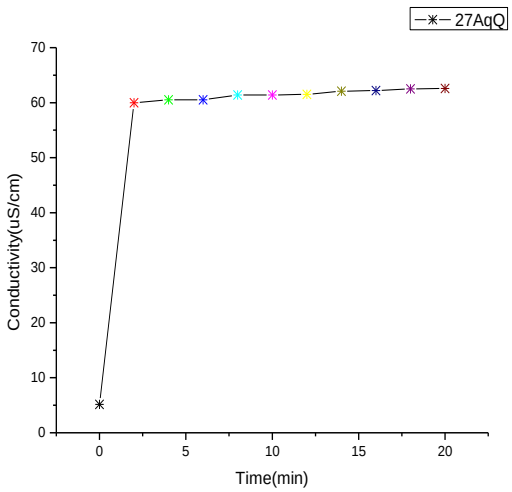


Figure 4.12: 27AqQ Conductivity Results

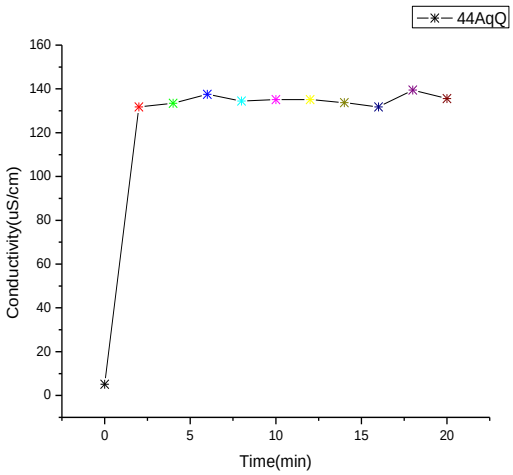


Figure 4.13: 44AqQ Conductivity Results

In Figures 4.12 and 4.13, a sharp increase in conductivity is observed from 0 min which quickly stabilises after 2 min. The conductivity values obtained lie in the safe zone of 0-800µS/cm (Tiffany, 2010).

4.3.7 Ultra Violet visible (UV/Vis) Spectroscopy

Visible light absorption is what causes objects to be coloured. It lies in the wavelength range 400–700 nm. When a material absorbs light, valence electrons are promoted from their grounded states to higher energy states.

Trace amounts of the biocide leached out of the fibres during the filtration process and this can be verified with the UV/VIS test results shown in Figure 4.14. The biocide has an absorbance peak at around 300 nm due to the abundant copper ions, and the water filtered with the three membranes does not show any absorbance peak. This further proves that the membranes can be safely used as water filters for human consumption.

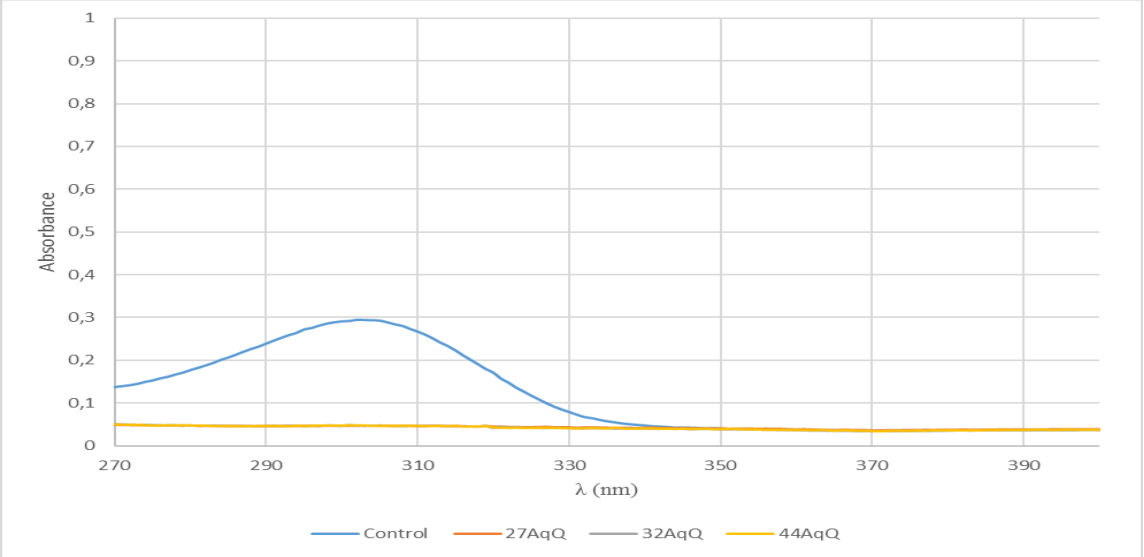


Figure 4.14: Absorbance Results of the 3 membranes

4.4 Conclusions

Antibacterial activity was observed for both the neat and biocidal nanofibers mats. The antibacterial activity of the neat nanofibers could be due to the polymer itself and also the solvent used during spinning which is isopropanol alcohol also known as rubbing alcohol. With increasing contact time of the bacterial cells and the membranes more antibacterial activity was observed and also the 32 mol. % neat or modified had the most antimicrobial efficacy. These results are not the expected because it was expected that the more hydrophilic the membrane, the more antimicrobial activity it will show. And for that reason, ICP-AES was done to determine the rate at which the metal ions are released from the membranes.

ICP-AES showed that the 44AqQ membrane had the fastest release rate as compared to the other membranes. 44AqQ being the most hydrophobic membrane, there is a possibility that the metal ions were on the surface rather than being dispersed within the nanofibers. The 27AqQ membrane showed the slowest release rate implying that there were chemical interactions retaining the metal ions within the polymer matrix.

In this first part of the study it was seen that the 27 mol. % EVOH neat or modified independent of contact time had the lowest antimicrobial activity. From BL Imaging it was seen that the nanofibrous mats are indeed antimicrobial by the loss of bioluminescence and also the percentage reduction in CFUs calculated. The percentage reduction varied from 56% to 99%. Zone inhibition tests further proved the antimicrobial properties of the fabricated membranes showing a large inhibition zone around the 44AqQ membrane. It was observed that the

nanofibrous mats, neat and modified were more effective in deactivating the gram-positive strain, *S. aureus* more than the *E.coli* bacterial strain.

Conductivity measurements and results obtained from UV/Vis spectroscopy showed that the amount or concentration of metal ions in the filtrate was negligible thus not harmful to the human body. This was a very important observation because this makes the fabricated nanofibrous membranes safe to be used for water sanitation for human consumption.

4.5 References

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4.6 Part B: Blending of Poly (ethylene-co-vinyl alcohol) and Polyethylene oxide as a way of enhancing the hydrophilicity of the nanofibrous membrane

4.6.1 Summary

In Part A of the study it can be seen that indeed the hydrophobicity of the nanofibrous membranes plays a major role in its use as an antibacterial water filter. To further investigate how the nature of the material surface has an effect on the use of the nanofibrous membranes in water sanitation application, EVOH was blended with Poly (ethylene oxide) (PEO). This was done to change its hydrophilicity. 27 mol. % EVOH was chosen for the experiments instead of the 32 mol. % or 44 mol. % because it was the one that was electrospinnable after being blended with PEO and produced comparable nanofibers.

Polymer blends have an advantage over copolymers or individual polymer systems in that they are not limited by suitable synthetic schemes. Therefore, nanofibrous mats formed by mixing different polymers become an appealing option, which is especially true for biofunctional polymers, as their chemical monomers are difficult to modify (Wang et al., 2009).

Polyethylene oxide is a crystalline thermoplastic, water soluble polymer with the general formula - $(CH_2CH_2O)_n$- where n can range from 4 to thousands depending on the grade. PEO is commercially available in a wide range of molecular weights from ethylene glycol, diethylene glycol and so on. The low molecular weight polymers which are hydroxyl terminated (Gombotz et. al.,) with n up to approximately 150 are generally known as polyethylene glycols and those with higher molecular weights are known as poly (ethylene oxide), polyoxyethylene or polyoxirane. Since PEO is water soluble, it is usually used for packaging making water soluble films. It also forms polyethylene oxide salt complexes which are used for alkali metal rechargeable batteries (Whelan, 1994).

This polymer's water solubility is of great interest considering that some polymers which have closely related molecular structures are insoluble in water e.g. polymethylene oxide or polyacetaldehyde. It has a low intrinsic toxicity that renders the polymer ideally suited for

biological applications (Schubert et al., 2010). It is also highly soluble in a range of organic solvents such as acetonitrile, chloroform and ethylene dichloride (Bailey et al., 1976).

4.7 Materials and Methods

4.7.1 Materials

Poly (ethylene-co-vinyl alcohol) (EVOH), (27 mol. % ethylene) was purchased from Sigma Aldrich in pellet form and Poly (ethylene oxide) in powder form with an average molecular weight of 1 000 000. Isopropanol/water (70:30 vol/vol) was used as the solvent. Merck supplied the isopropanol. AquaQure biocide was obtained from AquaQure water solutions, South Africa.

4.7.2 Preparation of electrospinning solutions

Different concentrations of the polymer blend were made up in order to find the right average diameter of the nanofibers to be used as filtration membranes. EVOH and PEO were dissolved in the isopropanol/water solvent to make up the solutions. When the right concentration was determined, the AquaQure biocide (5% vol/vol) was added to the polymer solution while stirring. This was all done at a temperature of 90 °C.

4.7.3 Electrospinning

Single needle electrospinning was employed in the fabrication of nanofibers. The horizontal setup used was designed in-house. The components are, a voltage supply unit (0-50 kV), a syringe pump, a spinneret that is connected to the positive terminal and a grounded collector plate connected to the negative terminal of the power supply unit. The voltage varied between 15 and 20 kV for the optimization experiments whilst the flow rate and tip to collector distance were maintained at 0.01 ml/min and 15 cm respectively.

4.8 Characterization of neat and biocidal EVOH/PEO nanofibers

SEM was done to see the morphology and measure the diameters of the nanofibers. FTIR was done to confirm the blending of the two polymers and DSC was done to see the melting and crystallisation behaviour of the polymer blend. ICP-AES was also done to see the release profile of the biocide from the membranes and lastly UV/Vis for the leaching tests.

4.9 Results and Discussion

4.9.1 Scanning Electron Microscopy

From SEM, the electrospun fibres from the polymer blend solutions were found to be in the nanometre range thus enabling a comparison study to be carried out between the 27 mol. % EVOH and the 27 mol. % EVOH/PEO blend. The average diameters are summarised in Table 4.5 below. The blend nanofibers are slightly larger than the 27 mol. % EVOH nanofibers due to the addition of PEO which increases the viscosity of the solution. (Angammana, 2011) reported that concentration and molecular weight indirectly affect the viscosity of the solution which is a significant parameter that influences the diameter and morphology of the fibre.

Table 4.5 Average fibre diameters of the non-blend and blend nanofibers

EVOH		EVOH/PEO	
Neat	**AquaQure**	**Neat**	**AquaQure**
310.5 nm	213.1 nm	461.9 nm	306.6 nm

As can be seen from the SEM images in Figures 4.15 and 4.16 respectively, there is not much visible beading in the fibres, a beautiful network of fibres was fabricated.

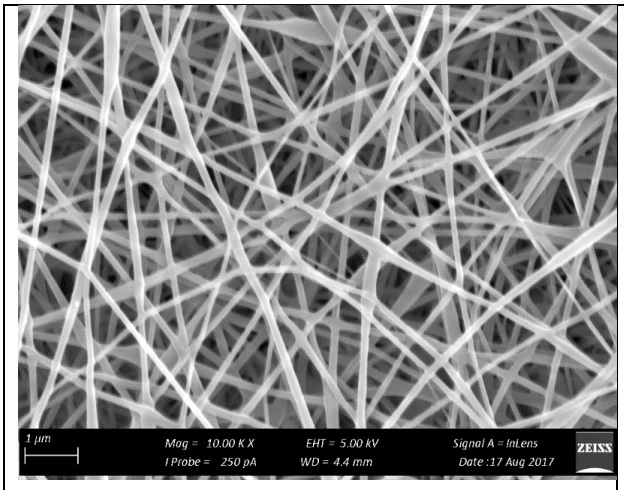


Figure 4.15: SEM Image of neat EVOH/PEO nanofibers

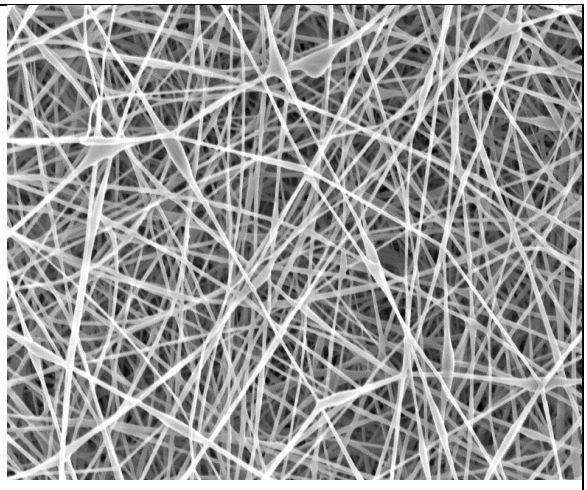


Figure 4.16: SEM Image of EVOH/PEO/AqQ nanofibers

4.9.2 Fourier transform infra-red spectroscopy (FTIR)

FTIR can be used to identify the interaction of polymer blends. A distinct chemical interaction such as hydrogen bonding exists between the chains if the two polymers are compatible, causing a change in the FTIR spectra. This change can be noted as band shifts and broadening (Jiao et al., 2007). In this part of the study, it was used to determine the successful blending of EVOH and PEO. Figure 4.12 below shows the individual FTIR spectra of EVOH, PEO, EVOH/PEO and EVOH/PEO/AqQ.

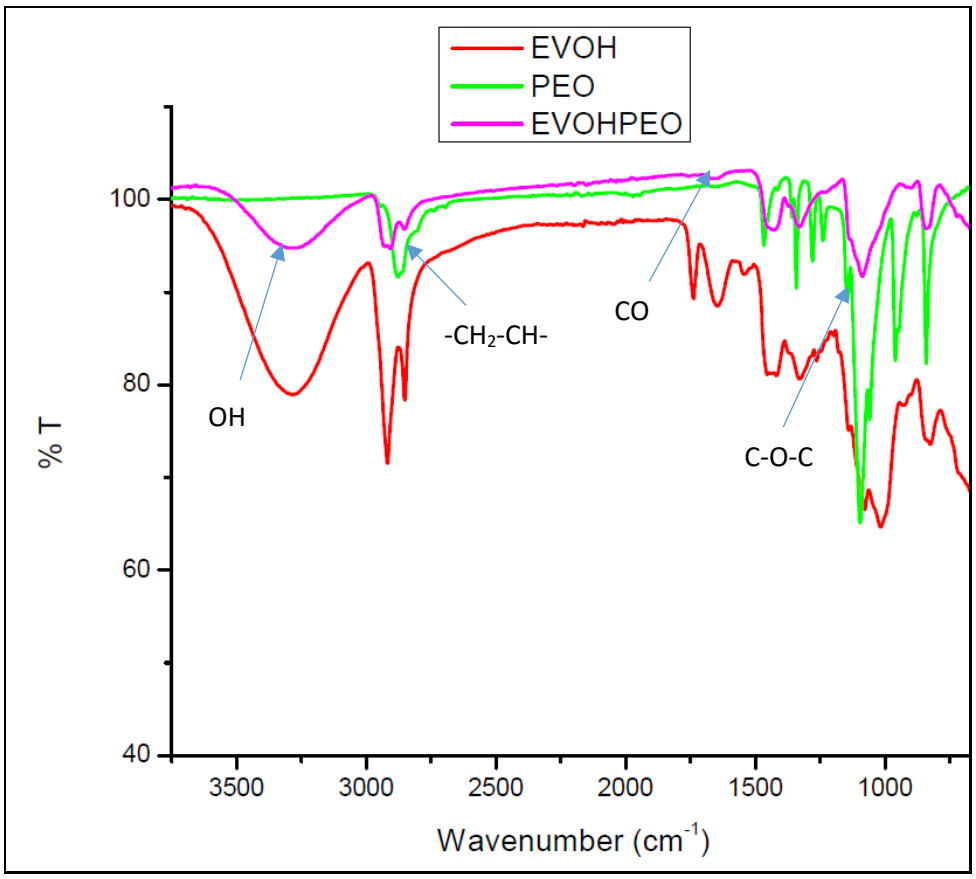


Figure 4.17: FTIR Spectra overlay of EVOH, PEO and the blend (EVOH/PEO)

EVOH's characteristic functional groups, the OH at 3250 cm^{-1} and the CO at 1625 cm^{-1} can be seen in the EVOH/PEO spectra. Overlapping of the -CH_2- and -CH- stretches in the (2850-2960 cm^{-1}) region from the EVOH and PEO polymers is observed. The ether, C-O-C (1050-1150 cm^{-1}) and C-O, alcohol (1000-1260 cm^{-1}) from the PEO as can be seen in Figure 4.17.This shows that the sample material tested was indeed a blend of the two polymers. There are notable shifts and disappearances of some peaks and this is due to the interaction between the EVOH and the PEO. When blending EVOH with PEO, the oxygen atoms in PEO can interact with the

hydroxyl groups in EVOH via strong hydrogen bond formation resulting in the broadening of the OH peak at shifts observed.

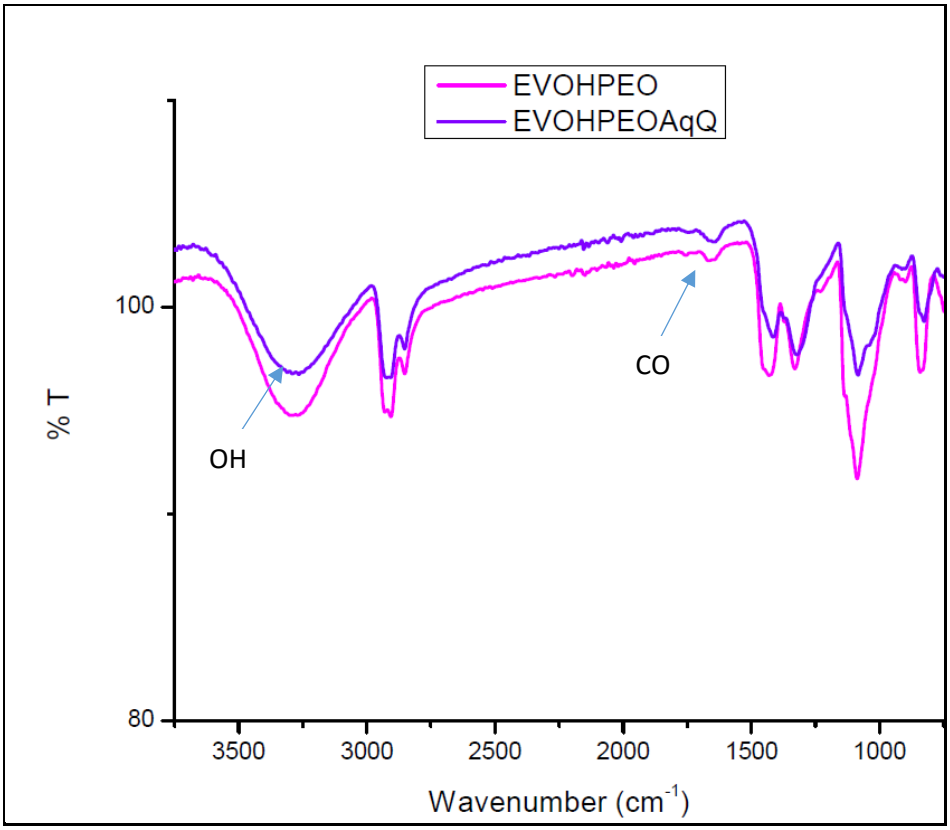


Figure 4.18: FTIR Spectra overlay of neat blend and AquaQure blend

Not much change in the spectra is observed after the addition of the biocide. Broadening and lowering of intensities of the OH, CO peaks as well as the CH_2 stretches can be observed. This is because of the chelating capabilities of the hydroxyl groups to retain the metal ions as discussed earlier. The low intensities indicate that the intramolecular and intermolecular interactions among the hydroxyl groups have been weakened (Li et al., 2010).

4.9.3 Differential scanning calorimetry (DSC)

Both EVOH and PEO are semi-crystalline materials and DSC was used to check the change if any, in thermal properties after the two polymers had been blended and also after the addition of the biocide. From DSC, EVOH had a melting peak at 187 °C and it crystallised at 165 °C. From the DSC thermograms in Figure 4.19 it can be seen that the two polymers are compatible and there is no phase separation because there is only one T_m and T_c peaks observed. The resultant T_m and T_c were 186 °C and 163 °C respectively. The PEO is reported to have acted

as spacers to enlarge the distance among the EVOH units and also formed hydrogen bonds with EVOH units, resulting in a physical network (Li et al., 2012). As a result of PEO's high molecular weight, less hydrogen bonds were formed in the network, and this may have weakened a little the hydrogen bonding effect resulting in lower T_m and T_c temperatures.

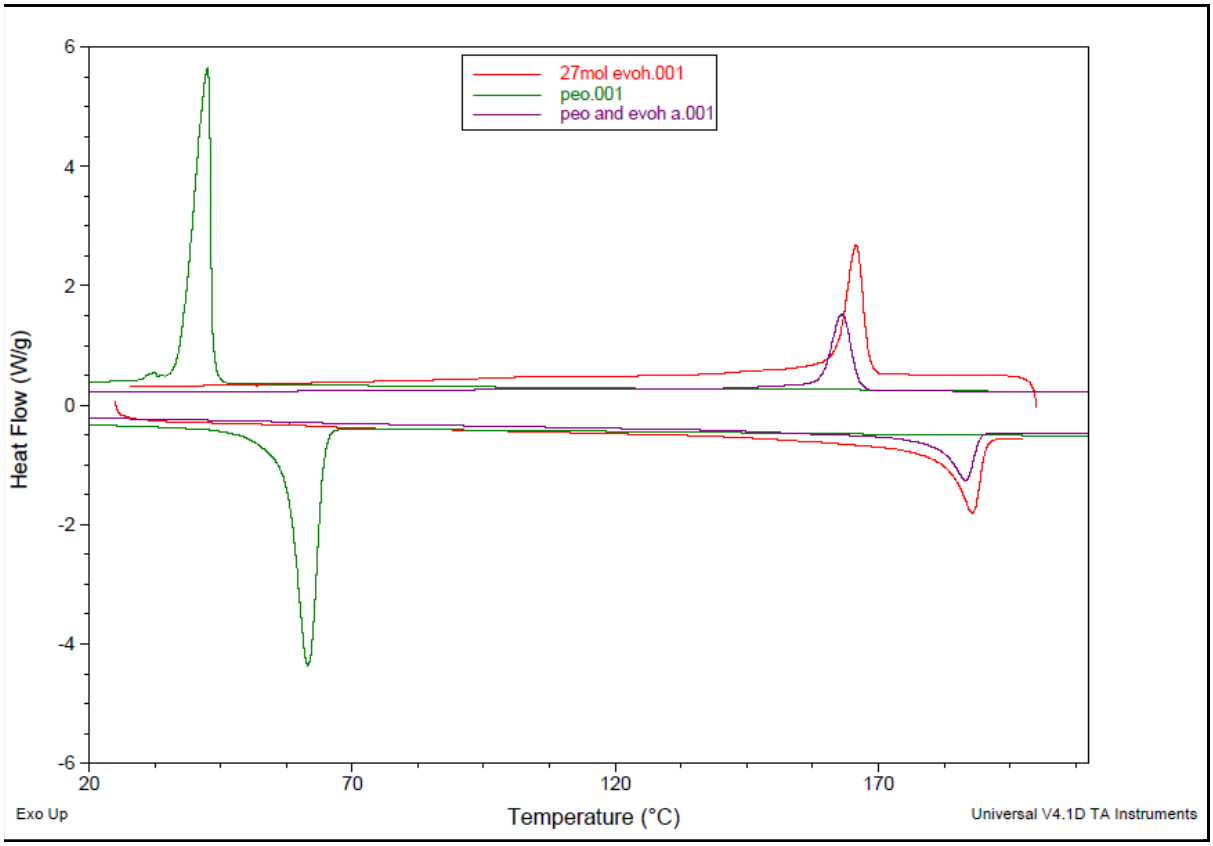


Figure 4.19: DSC thermograms of EVOH, PEO and EVOH/PEO

AquaQure was added to the polymer blend as these materials have a potential to be used as water filters. It is known from the first part of the study that the metal ions in the biocide interrupt the crystallinity of the EVOH matrix in a significant way. DSC confirmed that major changes in the crystallinity structure had occurred. Firstly after the blending with PEO as seen in Figure 4.19 above and then after the addition of the aqueous biocide solution. After the first cycle, no crystallisation nor melting was observed (Figure 4.20). It is possibly due to the plasticising effect of the PEO and the water (Finch, 1992) from the biocide since it is added as an aqueous solution. Degradation of the membrane started to occur at about 260 °C.

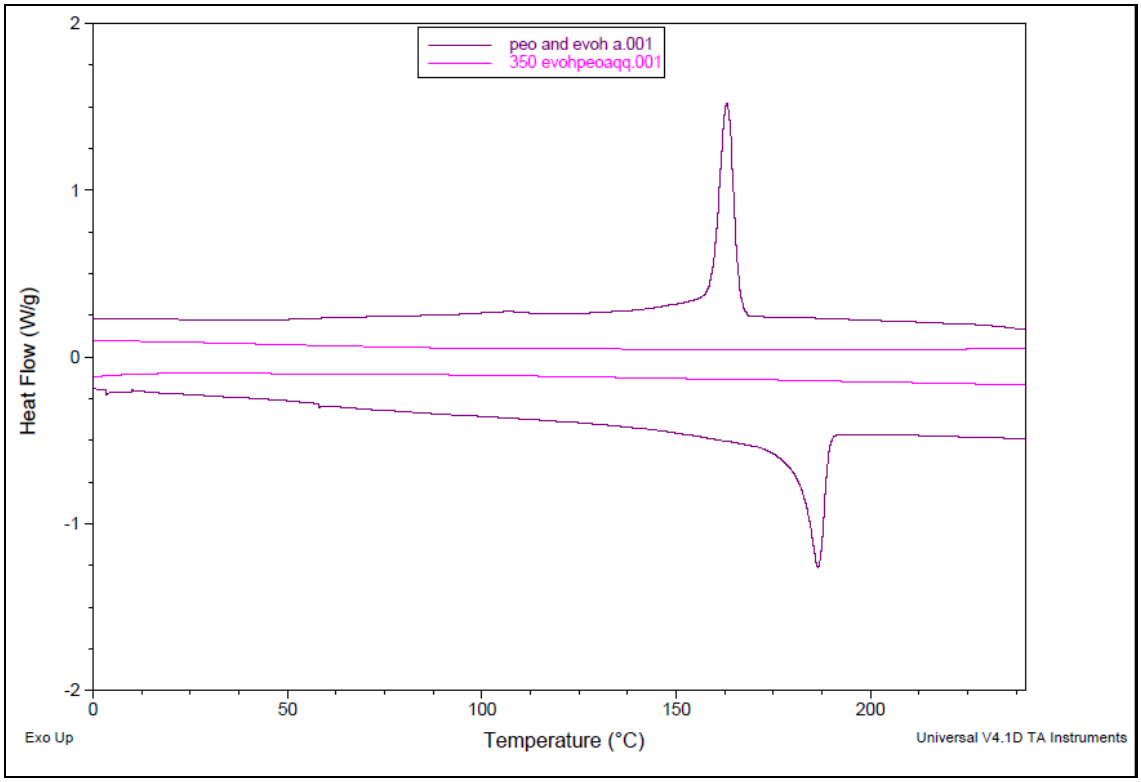
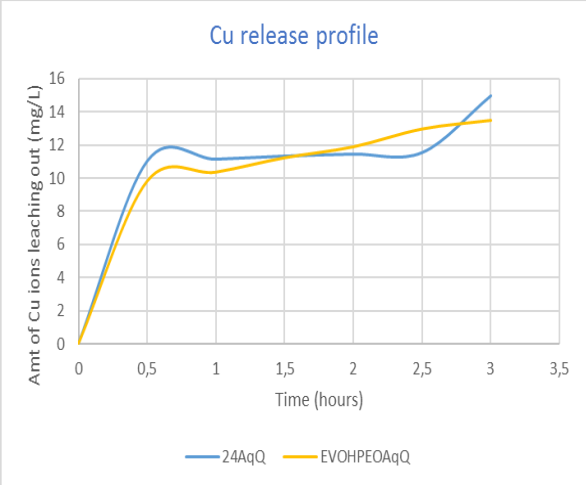


Figure 4.20: DSC thermograms of EVOH/PEO and EVOH/PEO/AqQ

4.9.4 Inductive Coupled Plasma-Atomic Emission Spectroscopy, ICP-AES (Release profiles)

For the membranes to be used in the intended application of water sanitation, the rate at which the biocide is released from the nanofibrous membranes is important. The two polymers, EVOH and PEO were blended as a way to investigate further the interaction of the metal ions with a more hydrophilic polymer system or matrix. Depending on the outcome of the release studies then further tests would be done.

From ICP-AES it was found that the rate of release of the biocide from the blend nanofibers was not much different from the EVOH nanofibers alone. The rate at which the Copper ions are released from the EVOH/PEO/AqQ (Figure 4.21) membrane is slightly lower than that of the non-blend membrane. The metal ions have more oxygen atoms to bond with therefore they are being retained within the polymer matrix. There is not much difference observed for the release of Zinc from the two materials. Copper ions are the most abundant in the biocide solution therefore they are the ones interacting more with the polymer thus being retained more within the polymer nanofibers.



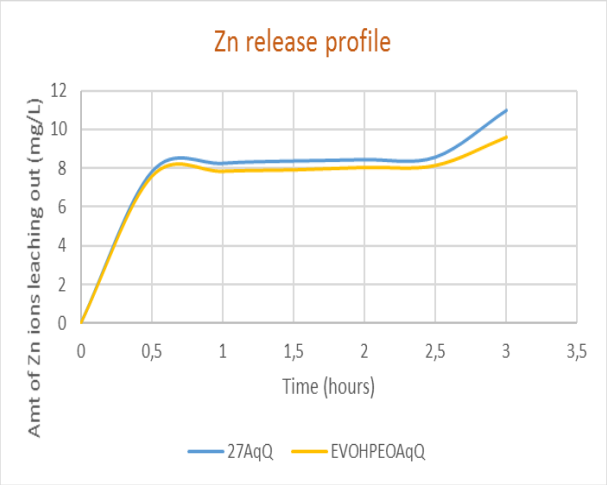


Figure 4.21: Copper release profile

Figure 4.22: Zinc release profile

From the results obtained from ICP-AES it was concluded that even if antimicrobial tests are conducted, there would not be much difference in the antimicrobial activity between the blend and no-blend membranes. Similar absorbance results are observed for the modified blend nanofibers as seen in Figure 4.23. The concentration of ions leaching out of the membrane a negligible.

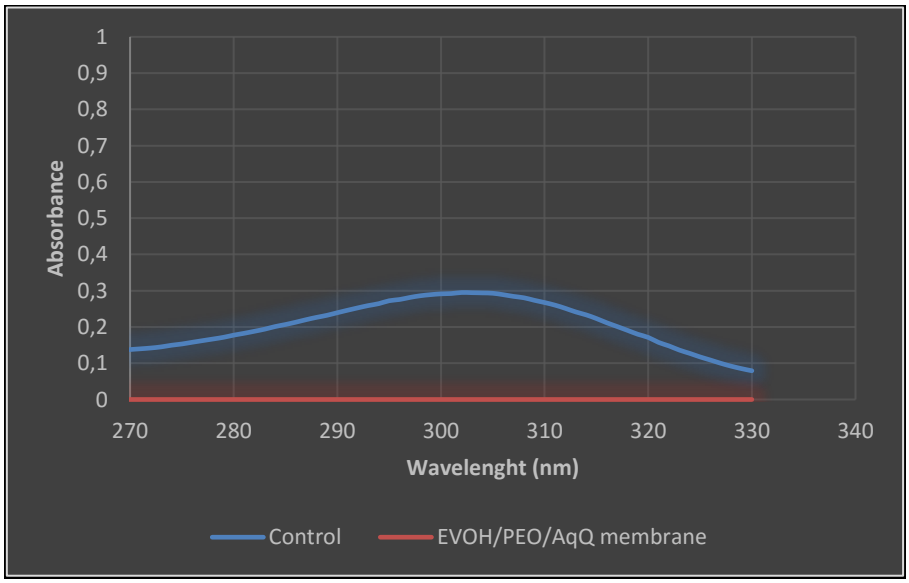


Figure 4.23: Absorbance Results

4.10 Conclusion

A polymer blend of Poly (ethylene-co-vinyl alcohol) and polyethylene oxide was successfully made. Single needle electrospinning was used to fabricate the nanofibrous membranes. Scanning Electron Microscopy was used to measure the fibres and they were found to be in the nanometre range. The EVOH/PEO neat fibres had an average diameter of 461.9 nm and the EVOH/PEO/AqQ fibres were 306.6 nm. This was a good average diameter because it allowed a comparative study to be done between the blend and the non-blend, EVOH alone. The nanofibers had no beading and a good network of fibres was produced as seen on the SEM images.

Fourier transform infrared spectroscopy confirmed the successful blending of the two polymers. The OH at 3250 cm^{-1} and the CO at 1625 cm^{-1} from the EVOH can be seen and the -CH_2- and -CH- stretches in the (2850-2960 cm^{-1}) region from both polymers. Though there was a lot of overlapping of the, C-O-C and C-O from the PEO observed. The hydroxyl shifted to lower wavenumber as expected after the addition of the biocide because of its chelating properties with the metal ions.

Differential scanning calorimetry showed that the two polymers were compatible since no phase separation indicated by two melting and crystallising peaks was observed. The addition of the metal ions had an unexpected effect on the crystallinity structure of the blend. The crystal structure was totally disrupted hence no crystallisation and melting was observed.

Inductive Coupled Plasma-Atomic Emission Spectroscopy was done to determine the rate at which the metal ions are being released from the material. It was seen that the copper and zinc were being released at a lower rate compared to the EVOH/PEO blend nanofibres. Since the blend was more hydrophilic, it was concluded that the metal ions interacted more with the blend components therefore being retained more within the matrix. These ICP-AES results led the study to the end in that since the release of the biocide was lower than that of the non-blend therefore even the antibacterial activity would not be much different. No absorption peak was observed for the water filtered through with the EVOH/PEO/AqQ membrane using UV/Vis spectroscopy.

Therefore to answer the research question**, Would the hydrophobicity of the nanofibers affect the properties of the material in water sanitation applications?** The answer based on the results obtained in this study is **YES** the hydrophobicity does affect the properties of the material in water sanitation applications. The materials behaved differently because of their different degrees of hydrophobicity as seen from the release studies. The more hydrophilic membrane did show an affinity for the metal ions rendering it not suitable to be used for this application. The nanofibrous membranes have to release the metal ions for them to be in contact with the bacteria resulting in the inactivation of the bacteria.

From the initial antibacterial tests, 32 mol. % EVOH proved to have the best antibacterial efficacy but as more tests were done, the 44 mol. % EVOH proved to be more effective for the intended application. Further investigations still need to be done to give a more definitive conclusion.

4.11 References

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Chapter 5

Conclusions and Recommendations

5.1 Summary

The aim of this study was to prepare and compare three types of poly (ethylene-co-vinyl alcohol) nanofibers and determine which type of fibre membrane would be most suitable for the application in water sanitation. A nano-biocide known as AquaQure was added to the polymer solutions to enhance their antimicrobial properties. Under this aim the specific objective was to investigate if the hydrophobicity of the nanofibers affects the properties of the material in water sanitation applications since the biocide is known to be an effective antimicrobial agent.

5.2 Conclusion

First and foremost electrospinning conditions had to be optimised. The solution concentration and voltage applied was varied. From the obtained measured average diameters, a polymer solution concentration of 5 wt. %, 15 kV applied voltage, 15 cm working distance and a flow rate of 0.010 ml/min were found to be the ideal electrospinning conditions. Under these conditions the nanofibers obtained, neat and modified had average diameters ranging between 200 and 330 nm. When the biocide was incorporated into the polymer solutions, the average diameters decreased. The aqueous biocide solution made the polymer system more conductive thereby thinning out the fibers.

The morphology of the fabricated nanofibers was analysed by SEM. The neat nanofibers showed little to no beading and the nanofibers with the biocide had no beading at all. In part B of Chapter 4 of the study, the blend fibers also showed no beading both the neat and the biocidal blend fibres. The average diameters obtained ranged between 300 and 400 nm. This was an acceptable range considering that the blending with PEO increased the viscosity of the polymer solution making it a bit difficult to electrospin under the same electrospinning conditions in part A.

To confirm the incorporation of the ions into the polymer matrices, FTIR was done since this is an easy and inexpensive tool to study the presence of specific functional groups in a molecule. From the spectra, the interaction of the ions with the polymer was determined. The hydroxyl peak in the EVOH shifted when the metal ion were introduced into the system. The OH groups have the ability to form complexes with ions via chelation. In the blend polymer

system of EVOH and PEO a lot of overlapping of peaks were observed such as the -CH_2- and -CH- stretches and the C-O-C and CO from the PEO.

Thermal stability of the fabricated membranes was studied using DSC and TGA. The thermal stability is important in that if need be for the membranes to be sterilised for reuse, the thermal behaviour has to be known. As the ethylene content increased form 27 mol. % to 44 mol. % the T_m and T_c decreased. This was ascribed to the reduction of hydrogen bonding within the polymer system because of less alcohol groups. In the blend nanofibers, there was a slight reduction in the T_m and T_c temperatures. Only single peaks were observed meaning that there was no phase separation and the blend was compatible. The crystalline structure was altered by the ions to form complexes with the hydroxyl groups. The T_m and T_c temperatures decreased quite significantly. In the EVOH/PEO/AqQ system, no T_m and T_c peaks were observed. The material was concluded to be amorphous. Three degradation steps were observed on the TGA thermograms of the modified nanofibers. This shows that the metal ions aid in the degradation of the membranes.

It is important that the membrane being used as a filter be water stable and not dissolve in water. The fabricated membranes showed integrity after being exposed to water for 48 hours. SEM images did not show any disintegration of the fibres except that they became flat.

Since the main objective of the study was to see whether the hydrophobicity of the membrane had an impact on its use as a water filter, static contact angles had to be measured. Even though we know that the higher the ethylene content, the more hydrophobic the polymer will be, static contact angles confirmed that. From the results obtained, we saw the contact angle increased after the incorporation of the biocide. 32AqQ had a contact angle of 116.55 which fell into the super-hydrophobic range.

The intended application for the membranes is water sanitation therefore antibacterial tests had to be conducted. CFU counting, BLI and zone inhibition tests showed that the fabricated membranes had antibacterial activity even the neat fibers. This is was expected because the biocide used is known to be effective at deactivating bacteria therefore release studies had to be done to determine the rate at which the ions leave the material. 44AqQ released the copper and zinc ions at a faster rate and this correlated with the zone of inhibition results. Comparing the release profiles of the 27AqQ and the EVOH/PEO/AqQ there was not much difference observed in fact that of the blend was lower than that of the non-blend. Leaching tests were done using conductivity measurement and UV/Vis. Trace amounts of ions were leaching out

into the water during filtration therefore it is safe to say that the membranes fabricated can be used for water filtration as an effort to do away with the conventional methods of water purification that involve the use of chemicals.

5.3 Recommendations for future work

- The dispersion of the metal ions within the polymer matrix has to be determined.
- More antibacterial tests need to be done using the blended membranes.
- More fundamental study of the three EVOH grades used to give more understanding of how their different properties affect their use as water filters.
- Biofouling studies on the different EVOH membranes to determine which will have the least biofouling.